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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 A β 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 A β 38, A β 40, and A β 42 in human plasma.

RESULTS—A β isoforms have a half-life of approximately three hours in plasma. A β 38 demonstrated faster turnover kinetics compared to A β 40 and A β 42. Faster fractional turnover of A β 42 relative to A β 40 and lower A β 42 and A β 42/A β 40 concentrations in amyloid positive participants were observed.

DISCUSSION—Blood plasma A β 42 shows similar amyloid-associated alterations as we have previously reported in CSF, suggesting a blood-brain transportation mechanism of A β . The stability and sensitivity of plasma A β 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 ($A\beta$), particularly $A\beta_{42}$, 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 $A\beta$ 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 $A\beta$ kinetics may precede imaging-based amyloid detection by many years (3). Decreased cerebrospinal fluid (CSF) $A\beta_{42}$ 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 $A\beta_{40}$ and $A\beta_{42}$ 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 $A\beta$ 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 $A\beta$ and have not been specific for AD pathology.

In order to understand the production, transport and clearance of $A\beta$, stable isotope labeling kinetics (SILK) studies of $A\beta$ demonstrated the half-life of $A\beta$ in the CNS is approximately 9 hours (10,11). A later study demonstrated that $A\beta_{42}$ kinetics in CSF are specifically altered with amyloidosis, with faster $A\beta_{42}$ turnover kinetics relative to $A\beta_{38}$ and $A\beta_{40}$ in amyloid positive individuals consistent with increased aggregation and deposition (3). Evidence supporting the transport of $A\beta$ across the blood-brain-barrier and through CSF suggests that 30–50% of plasma $A\beta$ originates from the CNS (12), and animal model BBB transporters of $A\beta$, RAGE and LRP, have previously been described (13,14). Understanding blood $A\beta$ transportation, concentrations and kinetics is paramount to a more comprehensive understanding of whole body $A\beta$ production, transport, and clearance between the brain, CSF and blood compartments. In this prospective study, we sought to determine blood $A\beta$ 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 $A\beta$ turnover in the blood in both amyloid positive and amyloid negative individuals and also report our findings of $A\beta$ 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 A β 42 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- $^{13}\text{C}_6$] 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- $^{13}\text{C}_6$] 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- $^{13}\text{C}_6$] 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 APP695 NL Cell Media Standards, Determination of plasma $^{13}\text{C}_6$ -leucine enrichment, and Immunoprecipitation of A β 38, A β 40, and A β 42

All targeted A β isoforms (A β 38, A β 40, and A β 42) were immunoprecipitated simultaneously from 2mL of plasma via a monoclonal anti-A β mid-domain antibody (HJ5.1, anti-A β 13-28) 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 20 μL of 100X protease inhibitor (Roche #11140920), 20 μL of 2.5% (w/v) Tween-20 (Sigma #P9416), 50 μL of 10X PBS (Sigma #P3813), and 100 μL of 5M Guanidine (Sigma #G4505). After pre-treatment, $2 \times 1\text{mL}$ aliquots from each corresponding collection time point were combined and spiked with 20 μL of a solution containing 3.75 pg/ μL $^{12}\text{C}^{15}\text{N}$ -A β 38, 25 pg/ μL $^{12}\text{C}^{15}\text{N}$ -A β 40, and 2.5 pg/ μL $^{12}\text{C}^{15}\text{N}$ -A β 42 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 μ L of neat formic acid (Fisher #A117-50) to elute A β 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 μ L acetonitrile and dried again *in vacuo* without heat to remove any residual formic acid. Each sample was then reconstituted in 50 μ L 100mM TEABC. Proteolytic digestion was initiated via the addition of a 50 μ L aliquot of 2.5ng/ μ L 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 2 μ L aliquot of 50% Trifluoroacetic acid (TFA, Sigma # T6508) and 100 μ L 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 150 μ L 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 $^{12}\text{C}^{15}\text{N}$ -A β 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 A β 38, 8.48% for A β 40, 6.81% for A β 42, and 5.73% for A β mid-domain. The linearity was demonstrated with r-squared values of >0.99 for each A β isoform (see supplemental figure 1).

LC-MS/MS analysis of A β peptides

Extracted digests were reconstituted with 25 μ L of 20 nM BSA Digest (Pierce #1863078) in 10% Formic acid/10% acetonitrile. A 4.5 μ L 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 μ L sample loop onto a Waters 100 $\times$ 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 A β 38, A β 40, and A β 42

In order to determine whether A β kinetics in the blood differs between amyloid positive and amyloid negative individuals, SILK time courses were obtained for plasma A β 38, A β 40, and A β 42. To determine plasma A β kinetic rates, isotopic enrichment ratios were calculated and plotted versus time to elucidate differences in the kinetics of A β isoforms in the blood. Notably, the half-life of the A β 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, A β 38 labeling kinetics peaked earlier and higher than A β 40 and A β 42, indicating a faster turnover rate. This pattern is unique to plasma A β kinetics and was not found in prior CSF A β SILK studies.

In prior CSF studies, A β 42 peaked earlier than A β 38 and A β 40 in amyloid positive individuals (3) indicating a faster loss of soluble A β 42 likely due to aggregation and deposition. In this plasma SILK study, the A β 38/A β 40 ratios were similar over time between amyloid groups (Figure 1B), indicating no difference in kinetic processing between A β 38 and A β 40. In contrast, the plasma SILK A β 42/A β 40 ratios demonstrated faster soluble A β 42 turnover kinetics in amyloid positive individuals (Figure 1C), as seen in prior reports of A β CSF SILK (2,3). While the average SILK A β 42/A β 40 ratio remained close to unity in the amyloid negative group, a drop after hour 12 in the A β 42/A β 40 ratio of the amyloid positive group indicates faster A β 42 turnover and aggregation in those with CNS amyloidosis (Figure 1C).

Absolute A β concentrations in human plasma

Human plasma samples were also analyzed for absolute concentrations of A β 38, A β 40, and A β 42 at each time point to investigate the production rates and whether CNS amyloidosis is associated with plasma A β differences. Average A β concentrations for the amyloid positive and amyloid negative groups for all time points was 27.40 pg/ml and 23.81 pg/ml (A β 38), 288.0 pg/ml and 272.4 pg/ml (A β 40), and 37.13 pg/ml and 30.13 pg/ml (A β 42), respectively. The A β 42 concentrations and A β 42/A β 40 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 A β 42/A β 40 throughout the study demonstrated similar values within the amyloid negative group. While A β 42/A β 40 differences were small between amyloid groups, they were statistically significant at most time points measured throughout the study (Figure 3A). An average of A β 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 A β 42/A β 40 ratios in the presence of amyloidosis is observed in CSF, which is hypothesized to be due to A β 42 concentrations decreasing before detection of accumulation by amyloid PET.

The magnitude of the difference in plasma is less than detected in CSF. While A β 42/A β 40 ratios in the CSF are decreased by approximately 50% in the presence of amyloidosis, in plasma A β 42/A β 40 ratios are decreased by 14.3% on average in amyloid positive relative to amyloid negative individuals (Figure 3B). Despite these relatively small differences in A β 42/A β 40 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 A β 42/A β 40 concentration ratios is illustrated in Figure 4, where both plasma and CSF A β 42/A β 40 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 A β 42/A β 40 is plotted as a function of PIB-PET MCBP, all amyloid positive individuals by PIB-PET had plasma A β 42/A β 40 below the threshold of 0.1243. However, there were several amyloid negative participants by PIB-PET who also demonstrated plasma A β 42/A β 40 ratios below this limit. This subset of participants might represent “false positives” when using plasma A β 42/A β 40 to detect amyloidosis. Alternatively, this finding may reflect the early alterations in A β 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 A β concentrations as biomarker for amyloidosis, a receiver operating characteristic (ROC) curve was generated from the averaged plasma A β 42/A β 40 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 A β 42/A β 40 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 A β 42/A β 40 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 A β 42/A β 40 concentration ratio as a tool for amyloid status classification (Figure 5).

DISCUSSION

For the first time, we report the kinetics of A β turnover in human plasma by applying SILK methods previously utilized to study A β metabolism in human CSF. We found that the half-life of A β 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 A β kinetics between the blood and CSF are evident with SILK analysis, including the faster turnover of A β 38 in the blood of both amyloid positive and amyloid negative participants. The cause for the faster metabolism of A β 38 may be due to faster clearance by peripheral clearance mechanisms in the liver or kidney or different transport rates.

The faster turnover of A β 42 relative to A β 40 is similar to alterations in CSF kinetics, but off a lesser magnitude. Consistent with a faster turnover rate, we found lower absolute concentrations of A β 42 and A β 42/A β 40 in the blood of amyloid positive individuals similar to findings in CSF. Taken together, these findings suggest that plasma A β 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 spectrometry.

The physiology of A β 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 A β 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 A β 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 A β transport mechanism which accounts for 30% to 50% of A β in blood (12). Further, A β amyloidosis occurs exclusively in the CNS, making a peripheral amyloidosis-induced blood A β alteration unlikely without a CNS source. Thus, we conclude that a major clearance pathway of brain A β 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 A β , and consequently, an important mechanism in the pathogenesis of AD. We propose the hypothesis that active and rapid transport of brain-to-blood A β 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 A β concentrations as a biomarker for diagnosis of AD and results have been conflicting. Most analyses found no association between plasma A β 40 and A β 42 levels and risk of AD (15); others note elevated levels of A β 42 in patients with MCI compared to cognitively normal controls and individuals with AD (16). Some prospective studies have shown that higher plasma A β 42 levels are associated with increased risk of sporadic AD, but such elevations occur just prior to the onset of clinical symptoms as A β 42 levels may subsequently decline as the disease progresses (17). However, these studies have not demonstrated a difference in plasma A β 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 A β 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 A β 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 A β 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 A β 42/A β 40 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 A β 42 concentrations and kinetics for CNS amyloidosis. Additionally, the multiple longitudinal measures within the same individual demonstrate stability and consistency of A β concentrations associated with amyloidosis. Limitations of this study include its relatively smaller population size (n=41) and measurement of PIB-PET scan or CSF A β 42/A β 40 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 A β measurements in this study suggest this A β 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 A β 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.

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Figure 1. Plasma A β SILK for A β 38, A β 40, and A β 42

(A): Average isotopic enrichment time course profiles normalized to plasma leucine for plasma A β 38 (blue), A β 40 (green), and A β 42 (red) (mean $\pm$ 95% CI) by labeling protocol (left: IV bolus; right: oral). Kinetic profiles of all three isoforms appear similar between labeling protocols, with A β 38 reaching its labeling peak before A β 40 and A β 42.

(B): Average isotopic enrichment ratios for plasma A β 38/A β 40 displaying both amyloid groups on the same plot (blue, amyloid negative; red, amyloid positive) (mean $\pm$ 95% CI) demonstrates similar rates of plasma A β 38/A β 40 turnover regardless of amyloid status or labeling protocol (left, IV bolus; right, oral).

(C): Average isotopic enrichment ratios for plasma A β 42/A β 40 displaying both amyloid groups on the same plot (blue, amyloid negative; red, amyloid positive) highlights the faster A β 42 turnover kinetics in the amyloid positive group (mean $\pm$ 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 $\pm$ 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 $\pm$ 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 A β 42/A β 40 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 $\pm$ 0.0033 in the amyloid negative group (blue) and 0.1111 $\pm$ 0.0019 in the amyloid positive group (red). This

reflects a 14.3% lower A β 42/A β 40 concentration in amyloid positive individuals compared to amyloid negative individuals overall (p value < 0.0001, mean $\pm$ 95% CI shown).

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Figure 4. Plasma A β 42/A β 40 concentrations correlate with two different measures of amyloidosis Plasma A β 42/A β 40 concentration ratios are lower in amyloid positive individuals (red) compared to amyloid negative individuals (blue) when amyloid status is determined by CSF A β 42/A β 40 concentrations. The relationship between decreased A β 42/A β 40 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 A β 42/A β 40 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 A β 42/A β 40 measurements, several participants classified as amyloid negative by PIB-PET were also found to have similarly low plasma A β 42/A β 40 values (below the threshold of 0.1243).

Figure 5. ROC curve

ROC curve analysis using average plasma A β 42/A β 40 concentration ratios over 24 hours demonstrates an AUC of 0.8865, indicating this assay has good accuracy as a diagnostic test to detect amyloidosis.