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Alzheimer's & Dementia 8 (2012) 295–303

Alzheimer's & Dementia

Effect of human cerebrospinal fluid sampling frequency on amyloid-β levels

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Abstract

Background: β-amyloid peptide (Aβ) is associated with neurodegeneration in Alzheimer's disease. Emerging evidence indicates that Aβ levels in cerebrospinal fluid (CSF) may serve as an early clinical biomarker for evaluating pharmacological activity of new drug candidates targeting Aβ production or Aβ clearance. Therefore, it is critical to understand whether intrasubject levels of CSF Aβ are consistent between sampling intervals to determine whether Aβ can be used as a pharmacodynamic biomarker for drug candidates. Previous studies have produced seemingly conflicting observations for the intrasubject stability of CSF Aβ levels; we attempt to reconcile these conflicting observations.
Methods: The current study examined the Aβ levels in CSF collected with various sampling frequencies from three clinical studies conducted in healthy young or elderly subjects at the same investigative site for the purpose of designing future studies.
Results: The results suggest that CSF sampling frequency and/or sampling volume contributes to intrasubject variability in CSF Aβ levels, and that lowering the CSF sampling frequency may help minimize this effect.
Conclusion: These results will help guide clinical trial design for Alzheimer's disease therapy.

Keywords: Alzheimer's disease; Biomarker; Cerebrospinal fluid; Amyloid; Clinical trial

1. Introduction

The hallmark of Alzheimer's disease (AD) at the microscopic level is an overabundance in the brain of senile plaques and neurofibrillary tangles, formed by abnormally folded amyloid-β (Aβ) and tau proteins, respectively [1–3]. The amyloid plaques are formed by peptides stemming from proteolytic cleavage of the amyloid precursor protein by β- and γ-secretases. The major species of Aβ are peptides of 1-40 amino acids (Aβ-40) and 1-42 amino acids (Aβ-42) [4]. Tau is a microtubule-associated protein predominantly expressed in nerve cells and is responsible for microtubule stabilization. Alterations in the amount of the tau protein or increases in the phosphorylation of tau can lead to the formation of neurofibrillary tangles [5]. Amyloid plaques, soluble Aβ peptides, and tau are all currently being investigated as targets for AD therapy.

Given the evidence supporting a pathogenic role for Aβ and tau in AD [4–6], many compounds in clinical development target the formation or clearance of Aβ or tau [7,8]. To date, the more advanced compounds in clinical trials have been those targeting the Aβ pathway. Many of these drug candidates, such as the γ-secretase inhibitor semagacestat, have failed in late-stage clinical trials owing to a lack of efficacy or safety concerns [9]. Additional clinical trials currently being run should clarify whether targeting Aβ remains a viable approach for the treatment of AD.

Because AD clinical trials that assess putatively disease-modifying therapies require a large number of participants treated for at least 1 year to determine efficacy, a rational

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doi:10.1016/j.jalz.2011.05.900

strategy is necessary to demonstrate that the compounds reach their targets and display the expected pharmacological activity [10–13]. For compounds that target the Aβ production and clearance pathways, cerebrospinal fluid (CSF) levels of Aβ may serve as a valuable biomarker for determining whether these drug candidates reach their target and display the expected pharmacological activities (for a review, please refer to Shaw [14] and Thal et al [15]).

Two recent studies, however, placed into question the utility of CSF Aβ as a pharmacodynamic biomarker. Bateman et al [16] investigated the stability of CSF Aβ levels over 36 hours. Their results suggested that intrasubject CSF Aβ levels varied significantly and tended to rise in a short term over 36 hours of serial sampling. The authors suggested that fluctuations of Aβ levels were dependent on time of day and activity [16]. Kang et al [17] reported that the fluctuations of Aβ levels in human CSF were similar to the diurnal fluctuations observed in the brain interstitial fluid of mice, and suggested that the sleep–wake cycle and orexin might play a role in Aβ levels. These data apparently conflict with data showing stability in CSF Aβ levels measured with isolated lumbar punctures over a 2-year period [18].

The increase in CSF Aβ levels over a 24-hour period currently makes it extremely difficult to use CSF Aβ as a biomarker to discern a drug effect over a short period. Therefore, if CSF Aβ is to be used as a biomarker for determining pharmacodynamic activity of new drug candidates, it is critical to identify the factors that can contribute to the fluctuation of CSF Aβ levels, so studies are designed to minimize this effect. One factor that may contribute to the fluctuations in CSF Aβ levels is the frequency of the CSF collections. We conducted three clinical studies to address this issue by measuring CSF Aβ levels in a single period and a two-period crossover study. The results suggest that sampling frequency and/or sampling volume is a contributing factor toward the rise in CSF Aβ levels.

2. Methods

2.1. Subjects

The three studies were conducted at PAREXEL/California Clinical Trials (Glendale, CA). Informed consent was obtained from all subjects. All subjects after screening were found to be in good general health and without neurologic disease. A lumbar catheter was placed immediately before CSF sample collection was started. The subjects were encouraged to stay in bed, and allowed free choice of when to sleep throughout the study. Each study was conducted on a different group of subjects, each using a different CSF sampling procedure. There were no subject dropouts or refusals. The sampling procedures and the demographics are shown in Fig. 1. In study A, six healthy young men (25–47 years old) participated, and 5 mL of CSF and 4 mL of blood were collected from each subject at 0 (6:00 A.M.), 1, 3, 5, 6, 7, 8, 10, 12, 14, 18, 22, and 26 hours. In study B, five healthy elderly men and two healthy elderly women (58–83 years old) participated, and 7 mL of CSF and 10 mL of blood were collected from each subject at 0 (5:30 A.M.), 1, 4, 8, 12, 18, and 24 hours. In study C, eight healthy young men (24–45 years old) participated and were divided into two groups. In one group of four subjects, 6 mL of CSF and 4 mL of blood were collected from each subject at 18 time points over 24 hours ("higher frequency" period) at 0 (8:00 A.M.), 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 16, 18, 20, 22, and 24 hours. Ten days later, CSF was collected from the same four subjects at seven time points over 24 hours ("lower frequency" period) at 0 (8:00 A.M.), 1, 4, 8, 12, 18, and 24 hours. In another group of four subjects, CSF was collected at lower frequency first followed by higher frequency CSF collection 10 days later from the same subjects. During each CSF collection in these three studies, the first 2 mL (corresponding to the tubing dead space) was voided. CSF aliquots of 250 μL were frozen at −80 °C immediately after collection in 1-mL siliconized polypropylene tubes.

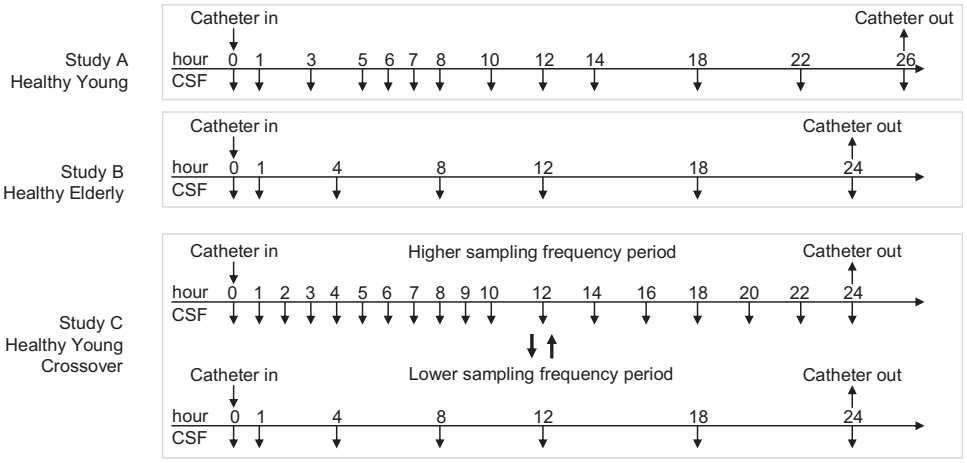
2.2. CSF analysis

$A\beta_{x\text{-}38}$, $A\beta_{x\text{-}40}$, and $A\beta_{x\text{-}42}$ were measured using human $A\beta_{x\text{-}38}/A\beta_{x\text{-}40}/A\beta_{x\text{-}42}$ multiplex assay (using 6E10 as detection antibody) by following the manufacturer's procedure (MesoScale, Gaithersburg, MD). To avoid interplate variation, all samples from each subject were measured together in duplicate on the same plate, and all the samples from the same study were measured on the same day. To avoid bias resulting from intraplate variation, all samples from each subject were randomized on the plate. The means of the intraplate coefficient of variation (CV) for the duplicates of samples and the standard calibrators included in the assay kit were less than 10%. The means of the interplate CV for the standard calibrators were less than 10%.

2.3. Statistical analysis

Initial examination of biomarker time curves showed variability, both between and within individuals. Because of the uncertainties associated with CSF collection, and the lack of a clear longitudinal model, it was decided to analyze area under the curve (AUC), which summarized each individual's time profile. The AUC provides a summary of biomarker levels that is unaffected by the presence of missing values and outliers. Because AUC results from an averaging process, the AUC is less variable than individual biomarker results. To the extent that the AUC captures the critical feature of the biomarker level, the predicted sample sizes should represent a minimum. Larger sample sizes would likely be required to power statistical tests associated with longitudinal analyses.

The mean and standard error were calculated for each subject at each time point using GraphPad Prism version 4.03 for Windows (GraphPad Software, La Jolla, CA). Sample size determinations were based on the rationale given in



GROUP	SUBJECT	MALE/FEMALE	MEAN AGE (SD)
Study A	Healthy Young	6/0	38.2 (8.2)
Study B	Healthy Elderly	5/2	64.8 (4.8)
Study C	Healthy Young	8/0	34.1 (7.6)

Fig. 1. The sampling procedures and the demographics in the three clinical studies. The horizontal arrow lines represent timeline. The down and up arrows above the timeline indicate the time points when the catheter was inserted and removed, respectively. The catheters were inserted immediately before the start of cerebrospinal fluid (CSF) collection, and removed at the end of the last CSF collection. The down arrows beneath the timeline indicate the time points when CSF was collected. Study A had 13 time points over 26 hours in six healthy young subjects. Study B had seven time points over 24 hours in healthy elderly subjects. Study C had crossover of two periods of CSF collections (indicated by vertical arrows) with a 10-day interval between the two periods. The higher frequency period had 18 time points and the lower frequency period had seven time point over 24 hours in eight healthy young subjects. Four of the subjects started with the higher frequency period followed by the lower frequency period, as opposed to the other four subjects.

Fleiss [19]. It is assumed that the implications of a change in biomarker CSF levels are appreciated and that a change in only one direction (either up or down) is of clinical interest. The sample size, n, required to detect a percent change in response, d, in a one-sided t test at 95% confidence with 80% power was obtained using the following approximate formula:

$$n=(z_{0.95}+z_{0.8})^2\frac{CV^2}{d^2}$$

where,

z_P = the Pth quantile of the cumulative normal distribution, and CV = % coefficient of variation of the change in response.

For a parallel group design, n represents the number of subjects in each treatment group. For a crossover design, where each subject is exposed to both treatments (e.g., placebo and drug) in different periods, n represents the total number of subjects needed in the study.

The AUC was taken as the area under the line that resulted from a simple linear regression of biomarker concentration (in pg/mL) on time (in hours). The integral was taken between 0 and 24 hours. Sixteen separate AUC responses were obtained from the two sample collection frequencies (low and high) from the eight subjects. The mean (μ) and standard deviation (σ) over the eight subjects were obtained separately for each CSF sampling frequency. The CV for an intergroup difference in AUC was taken to be $\sqrt{2}\sigma/\mu$.

The CV of the intrasubject 0-hour response difference required estimates of the period-to-period variance (σ_p^2) and within-period measurement variance (σ_m^2). These estimates, as well as the grand mean (μ), were obtained by variance component estimation from the 0-hour and 1-hour biomarker concentration measurements from each of the two collection periods for each of the eight subjects. For this purpose, the 32 results were considered as taken from a three-level hierarchal, nested structure (random time points within random periods and periods within random subjects). The CV for the intrasubject difference in the 0-hour measurement was taken to be $\sqrt{2(\sigma_p^2+\sigma_m^2)}/\mu$.

3. Results

3.1. Study A: Aβ levels in the CSF of healthy young subjects collected at 13 time points over 26 hours

Study A was conducted in which 65 mL of CSF was collected from six healthy subjects at 13 time points over 26 hours. The results showed that the level of Aβ in the CSF changed considerably relative to the first time point in all six subjects (Fig. 2A, B). On average, both $A\beta_{x\text{-}40}$ and $A\beta_{x\text{-}42}$ showed a pattern of a rise in levels over the first 14 hours relative to the first time point (Fig. 2C, D, respectively). Note that a small dip in average levels of both $A\beta_{x\text{-}40}$ and $A\beta_{x\text{-}42}$ is seen at 6 hours, and that this dip is primarily driven by one subject (subject D). The levels of $A\beta_{x\text{-}40}$ and $A\beta_{x\text{-}42}$ were significantly correlated with each other

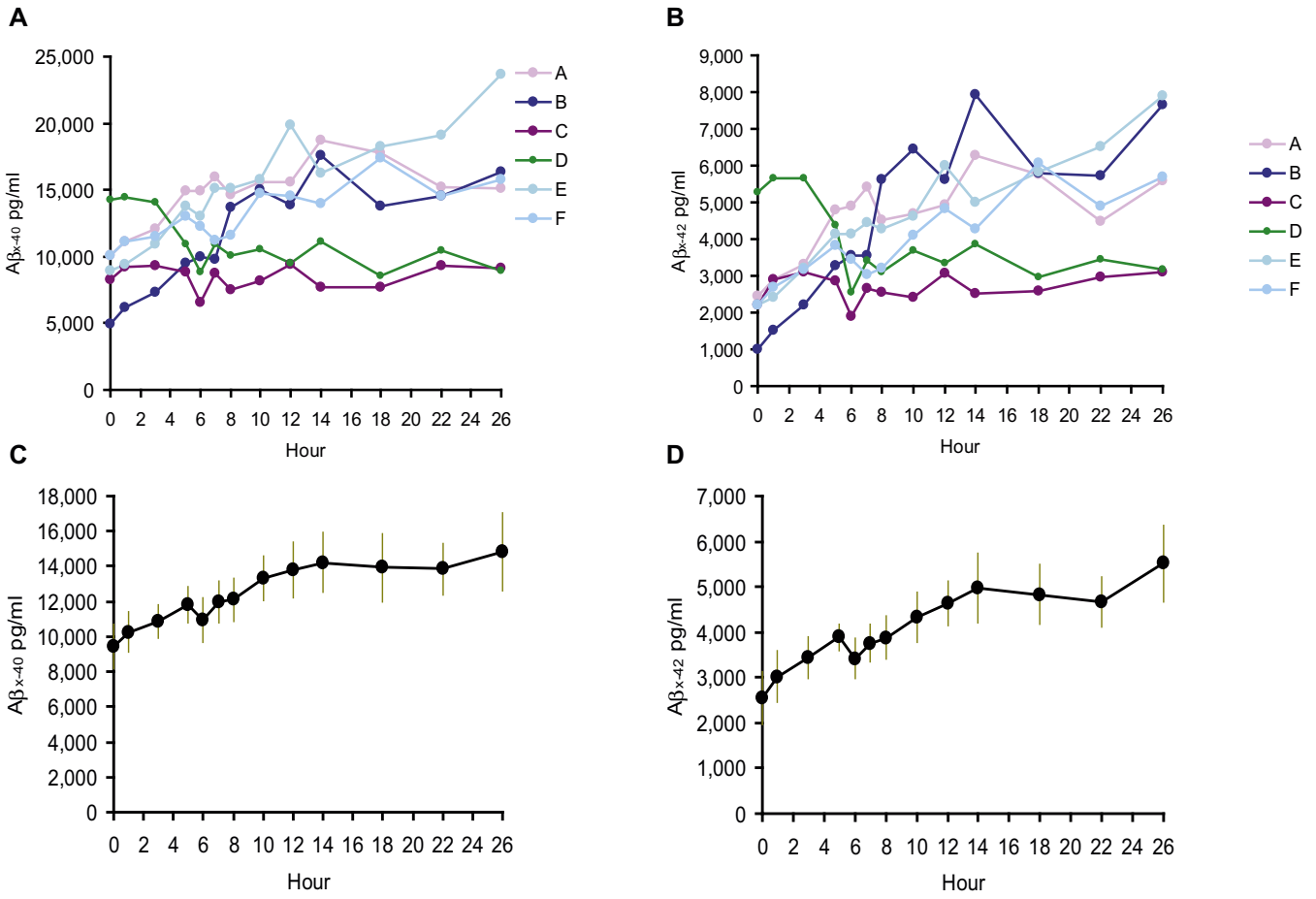


Fig. 2. Amyloid-β (Aβ) levels in the CSF samples collected at 13 time points over 26 hours in healthy young subjects (study A). $A\beta_{x\text{-}40}$ (A) and $A\beta_{x\text{-}42}$ (B) levels (in pg/mL) are shown at the 13 time points over 26 hours in the six healthy young subjects. Each color represents the same subject in the graphs of (A) and (B). The average $A\beta_{x\text{-}40}$ and $A\beta_{x\text{-}42}$ levels (in pg/mL) are shown at the 13 time points over 26 hours (C and D, respectively). Note that a small dip in levels of both $A\beta_{x\text{-}40}$ and $A\beta_{x\text{-}42}$ is seen at 6 hours, and that this dip is primarily driven by one subject (subject D).

(data not shown). Similar rises in the levels of $A\beta_{x\text{-}38}$ were observed (Supplemental Fig. 1A). On average, $A\beta_{x\text{-}40}$ and $A\beta_{x\text{-}42}$ levels started to rise at the beginning of the study and reached a steady state at 14 hours, which remained constant until the end of the sampling time course (Fig. 2C, D). The $A\beta_{x\text{-}40}$ and $A\beta_{x\text{-}42}$ levels at 26 hours were increased by 60% and 120%, respectively, above the baseline (0 hour) levels. In contrast, the levels of total protein and albumin did not change over the same period (data not shown), indicating that the rise in Aβ levels was not because of general changes in protein concentrations. Furthermore, $A\beta_{x\text{-}38}$ and $A\beta_{x\text{-}40}$ levels in the plasma did not show a general rise over 26 hours ($A\beta_{x\text{-}42}$ plasma levels were lower than the detection limit, data not shown), suggesting that the rise in CSF Aβ levels was centrally mediated.

3.2. Study B: Aβ levels in the CSF of healthy elderly subjects collected at seven time points over 24 hours

In this study, 49 mL of CSF was collected from seven healthy elderly subjects at seven time points over 24 hours. The results showed that in most of the subjects, there was no substantial rise in the levels of Aβ across the time points (Fig. 3A, B). Unlike study A, the average $A\beta_{x\text{-}40}$ and $A\beta_{x\text{-}42}$ levels at 24 hours were changed by only 13% and 15%, respectively, above the baseline (0-hour) levels (Fig. 3C, D). $A\beta_{x\text{-}38}$ levels also showed minimal change (Supplemental Fig. 1B). The levels of $A\beta_{x\text{-}38}$, $A\beta_{x\text{-}40}$, and $A\beta_{x\text{-}42}$ were significantly correlated with each other (data not shown).

3.3. Study C: Aβ levels in the CSF of healthy young subjects collected at higher and lower frequencies over 24 hours

To directly investigate the impact of CSF sampling frequency, a balanced crossover study was designed wherein the same subjects were subjected to CSF draws using a higher sampling frequency protocol and a lower sampling frequency protocol (study C, Fig. 1). Analysis of the CSF showed that during the higher frequency period, five of the eight subjects showed a rise in $A\beta_{x\text{-}40}$

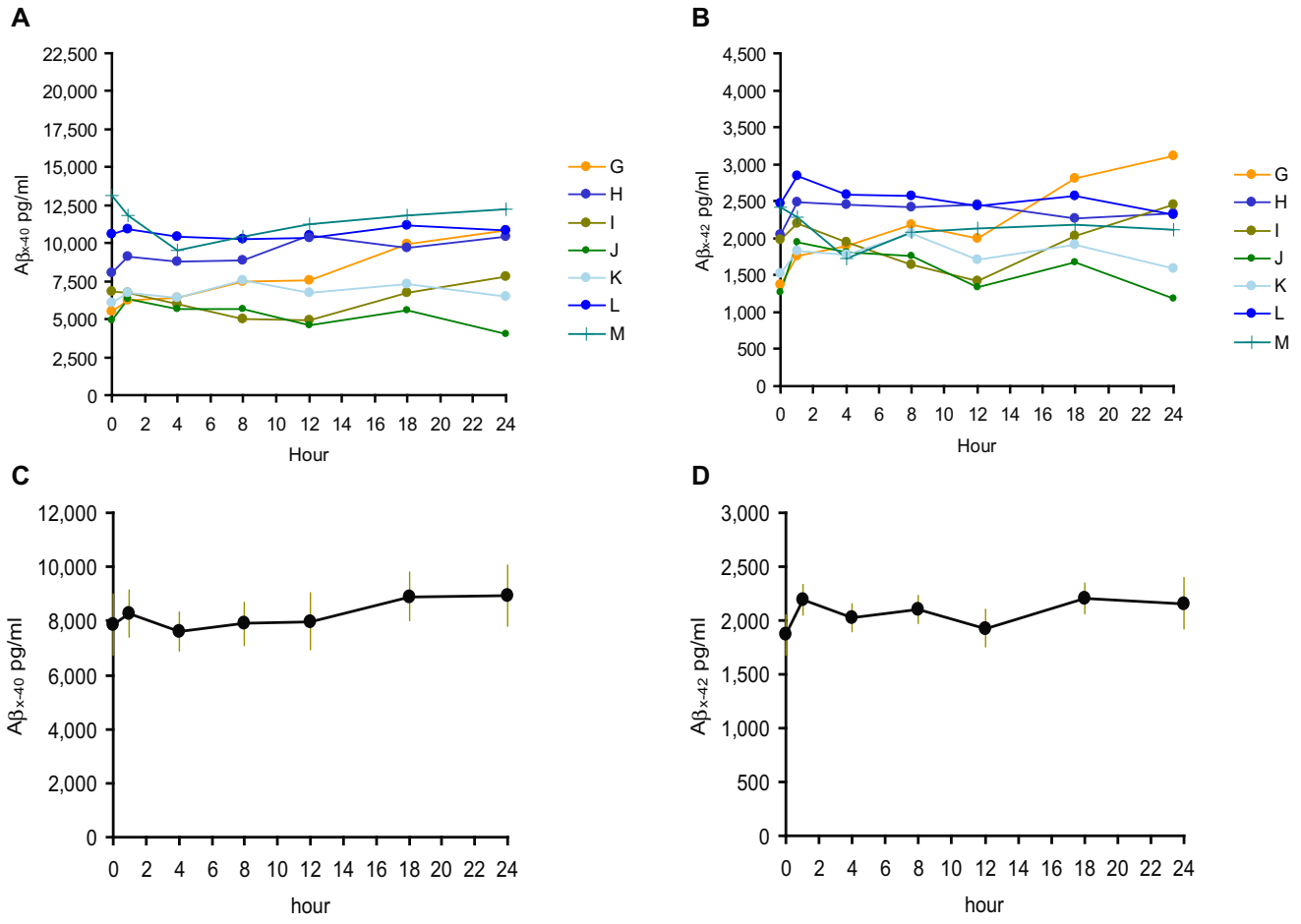
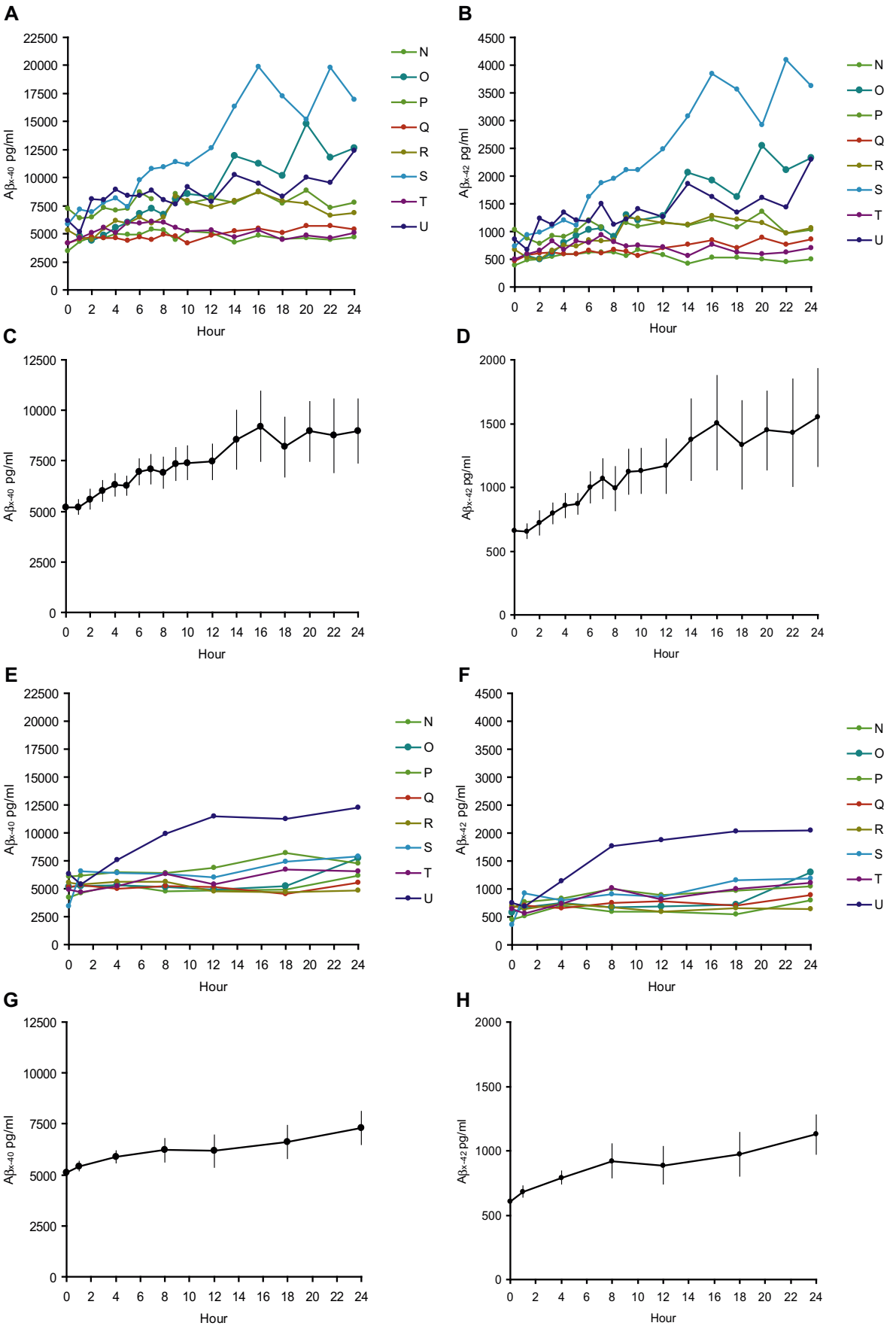


Fig. 3. Aβ levels in the CSF collected at seven time points over 24 hours in healthy elderly subjects (study B). $A\beta_{x\text{-}40}$ (A) and $A\beta_{x\text{-}42}$ (B) levels (in pg/mL) are shown at the seven time points over 24 hours in the seven healthy elderly subjects. Each color represents the same subject in the graphs of (A) and (B). The average $A\beta_{x\text{-}40}$ and $A\beta_{x\text{-}42}$ levels (in pg/mL) are shown at the seven time points over 24 hours (C and D, respectively).

and $A\beta_{x\text{-}42}$ levels over 24 hours relative to the first time point (Fig. 4A, B), similar to what was observed in study A. The average $A\beta_{x\text{-}40}$ and $A\beta_{x\text{-}42}$ levels at 24 hours are shown in Fig. 4C and D. Conversely, in the lower frequency period, only one of the eight subjects showed a substantial rise in $A\beta_{x\text{-}40}$ and $A\beta_{x\text{-}42}$ levels over 24 hours (Fig. 4E, F). The average $A\beta_{x\text{-}40}$ and $A\beta_{x\text{-}42}$ levels over 24 hours are shown in Fig. 4G and H. Similar results were obtained with $A\beta_{x\text{-}38}$ (Supplemental Fig. 1C, D). It is notable that three of the subjects reported having a headache, and they all showed a substantial rise in Aβ levels in the higher frequency period. Subject U, in particular, experienced both vomiting and headache in the higher frequency period. This was the only subject showing a rise in $A\beta_{x\text{-}40}$ and $A\beta_{x\text{-}42}$ levels in both the higher and lower frequency periods. The levels of total protein and albumin in the CSF samples of all the subjects did not change in the higher and lower frequency periods over 24 hours (data not shown), indicating that the rise in Aβ levels was not because of general changes in protein concentrations. The levels of $A\beta_{x\text{-}38}$, $A\beta_{x\text{-}40}$, and $A\beta_{x\text{-}42}$ were significantly correlated with each other (data not shown).

Figure 5 shows the comparison of $A\beta_{x\text{-}40}$ and $A\beta_{x\text{-}42}$ levels between the higher and lower frequency periods at the beginning (0 hour) and the end (24 hours) of a CSF collection period, or between the two sampling periods (day 1 and day 11) at 0 hour. At 0 hour, the Aβ levels showed relatively smaller intersubject variation and were similar between the higher and lower frequency periods (Fig. 5A for $A\beta_{x\text{-}40}$, B for $A\beta_{x\text{-}42\ 40}$). At 24 hours, in contrast, the Aβ levels showed relatively larger intersubject variation in the higher frequency protocol relative to the lower frequency protocol. When comparing the first CSF samples (0 hour) between day 1 (first period) and day 11 (second period), relatively small differences in $A\beta_{x\text{-}40}$ and $A\beta_{x\text{-}42}$ levels were observed (Fig. 5C, D, respectively).

To estimate the sample size required for a phase 1 clinical trial to detect changes in Aβ levels for an Aβ-lowering compound, based on the observed variability of Aβ values, a power analysis based on different intrasubject variability was conducted, as shown in Fig. 6. The results suggest that


A
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in a parallel design using the lower frequency CSF sampling protocol (seven time points over 24 hours), 13 subjects per group would provide sufficient power (with one-sided *t* test at 95% confidence and 80% power) to detect a 25% change in CSF $A\beta_{x\text{-}40}$ levels over 24 hours (Fig. 6A). In a study using the higher frequency CSF sampling protocol (18 time points over 24 hours), 28 subjects per group would be required to detect the same change (Fig. 6A). In a baseline controlled study with two CSF (pre- and postdosing) collections 10 days apart, four subjects would be needed to detect a 25% change at a single time point (Fig. 6A). For $A\beta_{x\text{-}42}$, approximately twice as many subjects per group would be needed to detect the same changes (Fig. 6B).

4. Discussion

To our knowledge, this is the first study to address the intrasubject variability of CSF Aβ levels in three human studies, in groups of healthy young or elderly subjects, with various CSF sampling frequencies, and at the same investigative site. A pattern of increasing CSF Aβ levels was observed using protocols with a higher CSF sampling frequency, similar to the previously published reports [16,17]. This rise was less evident in the protocol with lower CSF sampling frequency. These observations demonstrate that frequency or amount of CSF sampling may be a factor contributing to the intrasubject variability of CSF Aβ levels, and that lowering the CSF sampling frequency (or total sampled volume) may help minimize this effect. As such, these data contribute to our understanding of the increases in CSF Aβ seen over 24-hour collection periods reported in previous studies [16,17].

The pattern of a rise in CSF Aβ levels with repeated sampling over a 24-hour period has been observed by other investigators. Bateman et al observed that intrasubject CSF Aβ levels varied significantly and tended to rise over 36 hours of serial sampling [16]. A similar rise in CSF Aβ levels after multiple draws was observed by Tong et al [20]. The underlying reasons for the rise in Aβ CSF levels are not clear, but some factors that have been proposed to contribute include diurnal effects, time of day, and activity [16,17]. In our studies, the average Aβ levels did not return to the initial baseline level at 24 hours, suggesting that the rise was not caused by diurnal fluctuations.

The mechanism of the rise in CSF Aβ concentrations in catheter-based studies is not known. The data from this study suggest that the rise cannot be explained by endogenous factors alone, and may be related to the catheterization technique. It is possible that the pressure dynamics induced by the flow of CSF induces the rise in Aβ levels. This would be consistent with the previous observation demonstrating Aβ hyperproduction in settings of neural insult (for a review, please refer to Bissette [21]). It is also possible that Aβ is not homogenously distributed in the CSF, and high CSF sampling frequency alters the flow of CSF to redistribute Aβ to the lumbar subarachnoid space. In this regard, it is notable that subjects who had a headache were substantially more likely to have a rise in Aβ levels than those without this adverse event. This would suggest that low intracranial pressure and/or CSF redistribution may be mediating factors responsible for the rise in Aβ levels, but this theory needs to be confirmed by studies with a larger sample size. It is also important to note that the current study was not designed to differentiate between sampling frequency and total CSF volume sampled (108 mL and 42 mL of CSF, respectively, were collected in the higher and lower frequency periods in study C). Finally, one potential confounder for these studies is the unknown degree of leakage into the CSF space, which adds uncertainty to the precise volume of CSF obtained. For these reasons, it will be important to perform a more detailed assessment of the contribution of CSF sampling frequency versus volume and production versus clearance in the changes in CSF Aβ. In the latter case, the new approach developed by Bateman et al [22] to measure CSF Aβ production and clearance rates would be ideally suited to explore potential changes in Aβ production versus clearance in response to protracted lumbar catheterization. Finally, it is important to point out that other factors, such as disease state, may also contribute to any variability in CSF Aβ levels, which needs to be addressed by replicating these studies in patients with AD.

Pharmacodynamic biomarkers can be used to assess for evidence of target engagement or pharmacological activity of drugs in a phase 1 clinical trial, which can provide critical support for an early go/no-go decision in drug development. Given the intrasubject variability in Aβ levels in various clinical studies using different protocols, it is important to design a study that is able to detect a desired change of Aβ levels as an effect of a drug candidate targeting amyloid plaques or Aβ production and clearance pathways. The data from our study C allow for the determination of the number of subjects needed

Fig. 4. Aβ levels in the CSF collected at higher and lower frequencies over 24 hours in healthy young subjects (study C). In the higher frequency period, $A\beta_{x\text{-}40}$ (A) and $A\beta_{x\text{-}42}$ (B) levels (in pg/mL) are shown at the 18 time points over 24 hours in the eight healthy young subjects. The average $A\beta_{x\text{-}40}$ and $A\beta_{x\text{-}42}$ levels (in pg/mL) are shown in (C) and (D), respectively. In the lower frequency period, $A\beta_{x\text{-}40}$ (E) and $A\beta_{x\text{-}42}$ (F) levels (in pg/mL) are shown at the seven time points over 24 hours in the eight healthy young subjects, and the average $A\beta_{x\text{-}40}$ and $A\beta_{x\text{-}42}$ levels (in pg/mL) are shown in (G) and (H), respectively. Each color represents the same subject in all the graphs. Subjects U, S, Q, and O started with the higher frequency period followed by the lower frequency period, as opposed to subjects T, R, P, and N.

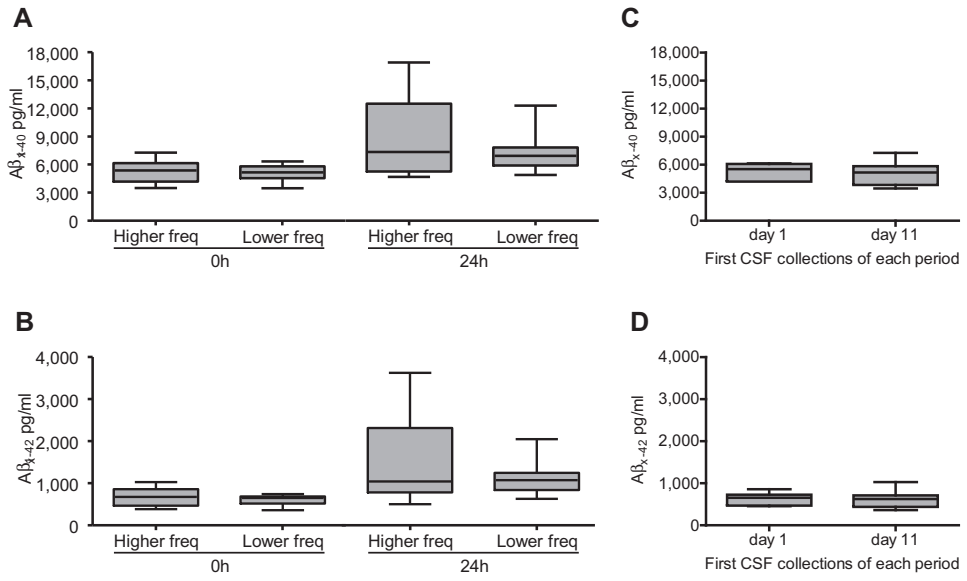

Fig. 5. Comparison of Aβ levels between the beginning and the end of CSF collection periods in study C. The average $A\beta_{x\text{-}40}$ (A and C) and $A\beta_{x\text{-}42}$ (B and D) levels (in pg/mL) are shown at the beginning (0 hour) and the end (24 hours) of the higher and lower frequency periods (A and B) of CSF collection, and the first CSF collections of the first period on day 1 and the second period on day 11 (C and D).

to detect a change in Aβ levels using different protocols for CSF collection. We found that decreasing the CSF sampling frequency resulted in a subsequent lowering of required sample size. A protocol that uses two individual lumbar punctures, spaced 10 days apart, provides the greatest power to detect a change in CSF Aβ levels. This is consistent with the recent report that intrasubject CSF levels of Aβ were stable at two time points separated by 2 years [18].

Taken together, the current study shows the effect of the CSF sampling frequency on intrasubject stability in CSF Aβ levels. These data are important for positioning CSF Aβ as a pharmacodynamic biomarker, and are critical for study design of clinical trials using CSF Aβ as a biomarker. Furthermore, even if CSF Aβ ultimately does not prove to be a viable pharmacodynamic biomarker for compounds targeting AD, these results may apply to any potential CSF biomarker, including tau, p-tau, soluble amyloid precursor protein, and beta-site APP cleaving enzyme. Further studies investigating the role of repeated CSF sampling on these potential biomarkers are currently underway.

Acknowledgments

The authors thank the volunteers for their participation in this study.

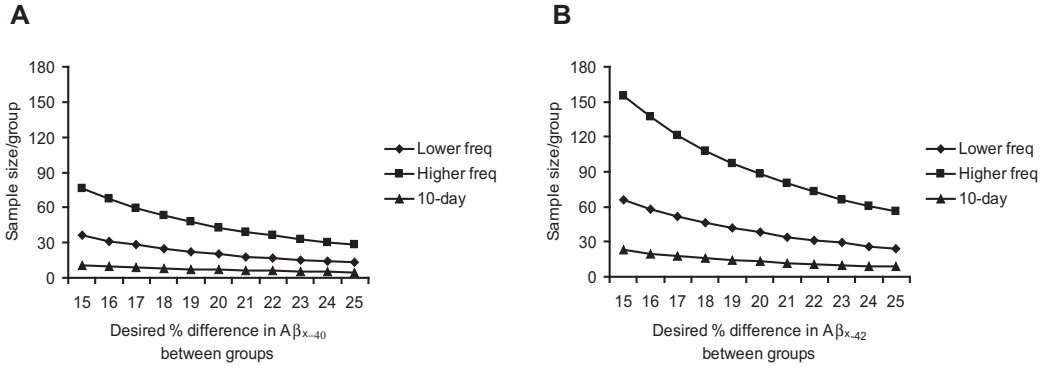

Fig. 6. Estimation of sample size for clinical studies detecting a change in Aβ levels. Power analysis based on different intrasubject variabilities in study C estimated sample size required for a desired percentage difference in $A\beta_{x\text{-}40}$ (A) and $A\beta_{x\text{-}42}$ (B) levels between groups in a parallel design with one-sided *t* test at 95% confidence and 80% power. The estimation was based on the study designs using the higher CSF sampling frequency protocol (18 time points over 24 hours, square), lower CSF sampling frequency protocol (seven time points over 24 hours, diamond), and two-period CSF collection protocol (pre- and postdosing time points over 11 days, triangle).

This study was funded by Abbott Laboratories. All authors are current or former employees of Abbott Laboratories or PAREXEL Early Phase Los Angeles.

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