Effect of spinal manometers on CSF amyloid beta concentration

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Running Title: Amyloid beta and spinal manometers

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Abstract

A laboratory simulation of lumbar puncture observed the possible effect on CSF Aβ concentration of using a manometer. Pooled human CSF samples were divided in two, one half passed through a manometer into a collection tube, the other transferred directly to a collection tube. CSF was analysed for $A\beta_{38}$, $A\beta_{40}$, and $A\beta_{42}$ using an electrochemiluminescence immunoassay. Use of a manometer decreased $A\beta_{42}$ concentration by 4.3% (± 2.4SE), $A\beta_{40}$ concentration by 4.4% (± 1.7SE), and $A\beta_{38}$ by 5.6% (± 1.5SE), relative to CSF not exposed to a manometer. The ratios of $A\beta_{42:40}$, $A\beta_{42:38}$ and $A\beta_{40:38}$ were not affected by manometer treatment. Factors which artificially lower CSF Aβ concentrations are of relevance to clinical diagnosis for AD and study design.

Key Words

Biomarkers, amyloid beta, cerebrospinal fluid, manometer, pre-analytical factor, Alzheimer's disease

Introduction

Amyloid beta (Aβ) peptides (particularly $A\beta_{42}$) are strongly implicated as an early driver of the sequence of neuropathological events thought to lead to Alzheimer's disease (AD) [1]. Due to their high diagnostic utility to early and late stage AD, analysis of CSF $A\beta_{42}$ and tau protein concentrations have become incorporated into the clinical diagnostic process, aimed at identifying AD patients during life [2][3]. Recent reports also suggest that ratios of Aβ peptides may improve detection of prodromal stage AD and better differentiate AD from other non-AD dementias over $A\beta_{42}$ alone [4–7]. Furthermore, concentrations of CSF Aβ peptides represent the targets and end points of many recent, and ongoing, clinical trials to develop AD therapeutics[8]. CSF is most commonly obtained by lumbar puncture. This involves inserting a spinal needle between the spinous processes of the lumbar vertebrae (typically L3 and L4), puncturing the dura mater, and entering the subarachnoid space. CSF then flows passively into a collection tube. During the collection of CSF, a manometer may be used to

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measure CSF opening pressure (the pressure of the CSF shortly after the subarachnoid space is breached), when the patient is in the lateral decubitus position. High (>25cm H_2O) and low CSF opening pressures (<6cm H_2O) [9] are seen in a range of different non-neurodegenerative conditions, and thus may inform differential diagnosis in the correct clinical context. Despite improvements in biomarker analysis over the last two decades, fluid-based biomarkers are subject to a number of potential confounding factors that can artificially alter the detectable concentration of proteins and other biomolecules. Confounding factors for Aβ have been extensively studied, and include: CSF collection technique [10,11], diurnal collection time [12], interval between collection and freezing [13–15], temperature [16–18], pH [19], sample matrix composition [20,21], bacterial infection [22], sample exposure to storage surfaces [23,24], and assay measurement variation [25–32]. Several papers have presented studies assessing multiple factors [33–36]. Bjerke *et al.* mentioned that two different catheters had no significant effect on $Aβ_{42}$ adsorption, but did not present any data or details on the experiment [33]. Here, we performed a new study to test the effect of passing CSF through a manometer on the concentration of Aβ peptides in a laboratory simulation of LP.

Materials and Methods

Collection and preparation of CSF

This study used de-identified CSF from patients of unknown disease status. Samples were collected by lumbar puncture, performed prior to 1pm, between the L3/L4 or L4/L5 inter-spaces; 10mL of CSF was collected at ambient room temperature into a 10mL polypropylene tube (Sarstedt, Nümbrecht, Germany cat. 62.9924.284) directly from the needle. Samples were centrifuged at 2200 Relative centrifugal force (RCF) for 10 minutes at 20°C, transferred to another 10mL tube (Sarstedt cat. 62.9924.284) and stored at -80°C within 1-4 hours of collection. CSF was thawed at 21°C for one hour and pooled into 20 unique 4mL samples and refrozen at -80°C.

Manometer simulation

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Pooled CSF samples were thawed at 21°C for one hour, each sample was divided into two 2mL aliquots in 25mL polypropylene (PP) collection tubes (Sarstedt, Nümbrecht, Germany, cat. 63.9922.254), designated 'Manometer CSF' and 'No Manometer CSF'. A volume of 1.5mL CSF Manometer CSF was manually ejected into a manometer made of styrene-butadiene copolymer (K-resin®) (Rocket Spinal manometer, Rocket Medical PLC, Washington, UK, order code:R55990, NHS SC Code: FTP002), using a 5mL pipette (Eppendorf PP 1-5mL graduated pipette tips; (Starlab, Milton Keynes, UK, cat. I1053-000). After 60 seconds of CSF injection, the manometer valve was released and the CSF allowed to drain into a 25mL collection tube (Sarstedt cat. 63.9922.254). The approximate inflow rate of CSF through the manometer during the experiment was ~0.04 cm^3/s (diameter = 4mm, velocity = 21cm/minute), similar to what would be expected during LP with an opening pressure of 21cm H_2O. 1.5mL CSF from the No Manometer CSF tube was ejected by pipette directly into a 25mL over the course of 60 seconds. Aliquots of 0.5mL Manometer and No Manometer CSF were created in 2mL tubes (Sarstedt cat. 72.694.406) and frozen at -80°C.

Electrochemiluminescent immunoassay analysis

CSF was analysed in duplicate plate wells (randomised, double blind order) for Aβ peptides $Aβ_{x-42}$ ($Aβ_{42}$), $Aβ_{x-40}$ ($Aβ_{40}$) and $Aβ_{x-38}$ ($Aβ_{38}$) using Meso Scale Discovery V-PLEX Amyloid beta peptide kit (6E10), on a Meso Scale Discovery SECTOR 6000 (Meso Scale Discovery, Rockville, Maryland, USA). Two assays (Assay 1 and Assay 2) were conducted on different days. Assay 1 analysed Manometer and No Manometer samples 1-10 and Assay 2 analysed Manometer and No Manometer samples 11-20. Assays were conducted according to the manufacturer protocol.

Statistical analysis

Microsoft Excel (Microsoft Office Professional Plus 2010, version: 14.0.7172.5000) was used to generate descriptive statistics and graphs. A two tailed, paired t-test in Excel (alpha 0.05) was used to compare Manometer CSF and No Manometer CSF results. Data for each Aβ peptide was normally distributed (D'Agostino-Pearson test $Aβ_{42}$: $p = 0.8$, $Aβ_{40}$: $p = 0.9$, $Aβ_{38}$: $p = 0.9$).

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Results

Variability of assays

Intra-assay variability was <3%CV in both assays for all peptides. Inter-assay variability was assessed by an internal quality control CSF sample ($A\beta_{42}$ = 6.7 %CV, $A\beta_{40}$ = 9.5 %CV, $A\beta_{38}$ = 3.8 %CV) according to International Organization for Standardisation standards [37].

Effect of manometer

Relative to No Manometer CSF, Manometer CSF Aβ concentration was decreased by $A\beta_{42}$: 4.3% (± 2.4 SE), $A\beta_{40}$: 4.4% (± 1.7 SE), and $A\beta_{38}$: 5.6% (± 1.5 SE) (Figure 1). A paired t-test showed that this was statistically significant in all peptides - $A\beta_{42}$: p = 0.047, $A\beta_{40}$: p = 0.026, $A\beta_{38}$: p = 0.002 (Table 1). Comparison of the ratios $A\beta_{42:40}$, $A\beta_{42:38}$, and $A\beta_{40:38}$ revealed no significant differences between Manometer CSF and No Manometer CSF samples ($A\beta_{42:40}$: p = 0.626, $A\beta_{42:38}$: p = 0.896, and $A\beta_{40:38}$: p = 0.158, see Table 1).

Discussion

During a routine LP procedure, a manometer may be employed to measure the opening pressure of CSF. Our findings suggest a decrease of 4-6% for $A\beta_{38/40/42}$ after CSF passes through a Rocket Spinal Manometer, made of styrene-butadiene copolymer (K-resin®). To our knowledge this material has not previously been studied in terms of its interaction with Aβ peptides. This small concentration change would seem unlikely, by itself, to greatly influence diagnosis of AD in individuals attending clinic.

However, the effect may be more relevant to clinical trials for AD therapeutics, where altered Aβ concentration is often a secondary endpoint, as use of large datasets does not compensate for such forms of systematic error [38]. Sampling procedures in cross-sectional studies where the use of manometers has not been standardised may result in biased biomarker profiles between cohorts. For

example, cognitively normal control samples continue to be challenging to acquire in large numbers, and AD focused collaborations may share sample cohorts without shared collection protocols. Furthermore, inconsistent use of manometers in longitudinal studies could raise levels of residual variation (statistical 'noise') in intra-individual biomarker data, which may obscure a real change, e.g., in a clinical trial; or create a bias if baseline CSF were to be taken without a manometer, but subsequent follow-ups used one. In either scenario, lack of standardisation in manometer use could mislead, or obscure, therapeutic effect in the region of 5-10% difference between comparators. These results are likely to be relevant to catheters, which are used in time-course studies of CSF Aβ concentration in trials assessing physiological variability or target engagement [11,39–41], though material and dimensions may alter the degree of Aβ concentration change. Finally, we found that the ratio of Aβ peptides was unaffected by manometer treatment, due to similar degree of treatment-dependent protein loss between each peptide measured. This provides a further reason to consider the use of Aβ ratios as diagnostic biomarkers for AD [4–7,42]. Our data supports the implication that an Aβ ratio may be useful in routine clinical diagnosis from the perspective of controlling for pre-analytical variation.

The experiment was a simulation of LP procedure conducted in the laboratory. An advantage of this was the ability to closely control the conditions of the experiment, for example removing the potential bias in CSF gradients from sequential tapping CSF with and without a manometer. However this approach also had limitations in fully capturing the circumstances of a real LP procedure. The dimensions and material of a pipette tip (polypropylene) differ from those of a lumbar needle (often stainless steel with a hub that can be made of metal or polypropylene). Additionally, CSF was stored at 21°C prior to contact with the manometer, whilst CSF collected during LP would be at approximately ~37°C, pH 7.33, decreasing and increasing rapidly respectively, once outside the body [43]. Finally the sample size of the study was small (n=20), and conclusions drawn from it would benefit from independent replication.

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In summary, this study revealed a small, significant, decrease in $A\beta_{38/40/42}$ when CSF was exposed to a spinal manometer. Ratios of Aβ peptides were unaffected. Ongoing and future trials measuring CSF opening pressure would be well served to consider the implications of this in study design.

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Figure 1

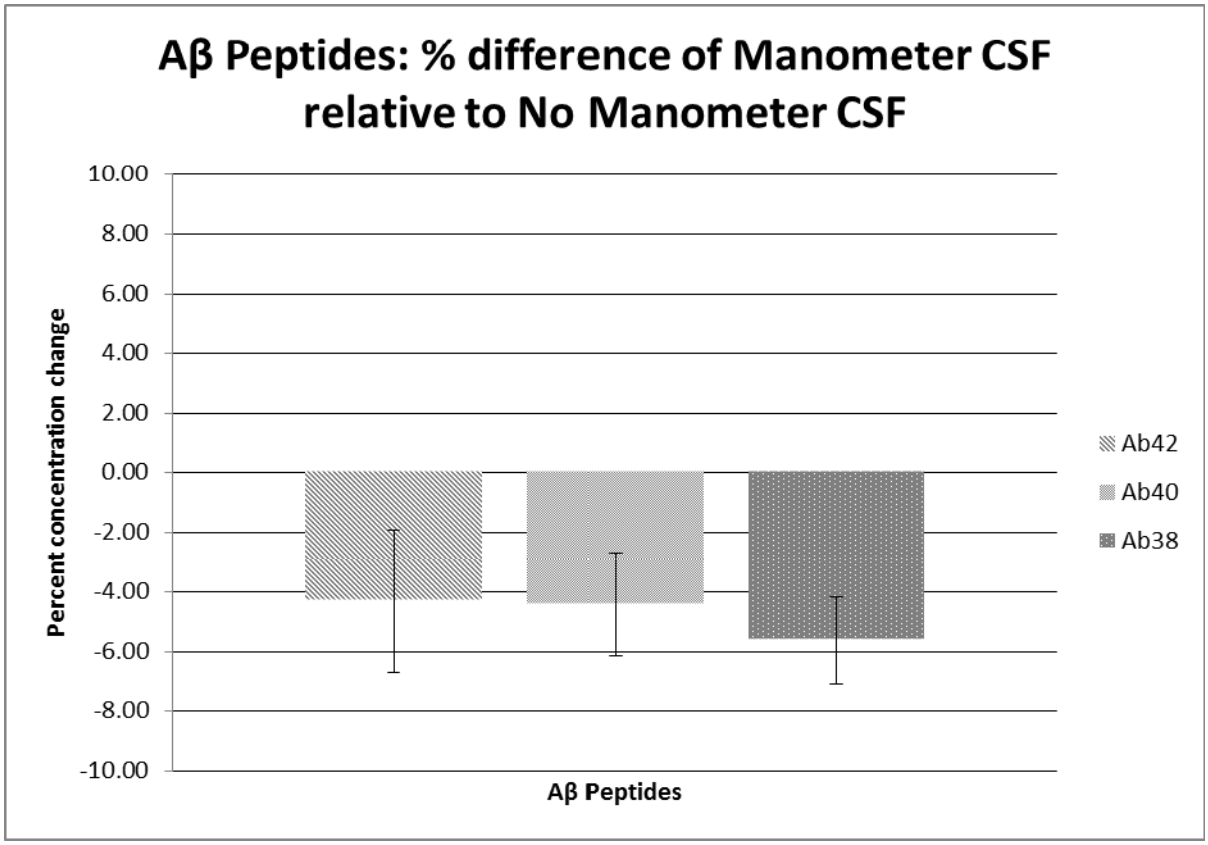

Figure 1: Showing the percent difference of Aβ peptide concentration in CSF pipetted through a manometer relative to the same CSF merely pipetted into a collection tube. All Aβ peptide concentrations tested decreased with manometer use. Error bars represent standard error.

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Table 1

A **B**

Table 1: Electrochemiluminescent assay results for Aβ peptide concentration in A) CSF pipetted directly into a collection tube (No Manometer CSF) and B) CSF passed through a manometer (Manometer CSF). Table 1B includes the results of a paired, two tailed t test based on the data for each peptide in tables 1A and 1B. Results show statistically significant decrease in Aβ peptide concentration in CSF given the manometer treatment, and no statistical difference in the ratio of Aβ peptide between sample treatments.

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