LUMBAR DRAINS CAN AFFECT CSF BIOMARKER LEVELS

Claudia L Craven MRCS MSc [1] claudia.craven@gmail.com
Miles D Chapman PhD [5] miles.chapman@uclh.nhs.uk
Linda D’Antona MBBS BSc [1] linda.D’Antona@uclh.nhs.uk
Simon D Thompson MSc [1] simon.thompson3@nhs.net
Henrik Zetterberg PhD MD [2,3] henrik.zetterberg@clinchem.gu.se
Laurence D Watkins FRCS (Neuro.Surg.) MD [1] laurence.watkins@uclh.nhs.uk
Ahmed K Toma FRCS (Neuro.Surg.) MD (Res.) [1] ahmedtoma@nhs.net

[1] Victor Horsley Department of Neurosurgery, National Hospital for Neurology and Neurosurgery, Queen Square, London, WC1N 3BG, UK
[2] Department of Molecular Neuroscience, UCL Institute of Neurology, Queen Square, London, WC1N 3BG, UK
[3] Department of Psychiatry and Neurochemistry, Institute of Neuroscience and Physiology, the Sahlgrenska Academy at the University of Gothenburg, S-431 80 Mölndal, Sweden
[4] UK Dementia Research Institute at UCL, London, WC1N 3BG, UK
[5] Department of Neuroimmunology, National Hospital for Neurology and Neurosurgery, Queen Square, London, WC1N 3BG, UK

Corresponding author: Claudia L Craven
Postal Address: Victor Horsley Department of Neurosurgery, National Hospital for Neurology and Neurosurgery, Queen Square, London, WC1N 3BG, UK.
Email: claudia.craven@gmail.com
Phone: +44 7454336888

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ABSTRACT

We investigate the effect of both silver-lined (Silverline®, Spiegelberg, GMBH & Co.) and barium-impregnated (EDM, Medtronic) lumbar catheters on concentrations of Aβ1-42 and T-tau in CSF.

CSF was collected from individuals of unknown disease via lumbar puncture (LP). CSF was thawed at 21°C CSF and and centrifuged for 5 minutes. A volume of 800uL was injected into (1) 2ml Sarsted aliquot (control), (2) barium-lined LD 80cm (3) silver-lined LD 80cm. CSF was collected through an LD over 3 different time periods: 30 seconds, 1 minute and 5 minutes (drain clamped before allowing CSF to drain). CSF was then collected into 2ml Sarsted aliquots. Each test was repeated three times per tube type, and per time protocol. Mean CSF concentrations (pg/ml) were corrected for total protein.

A maximum reduction of Aβ1-42 pg/ml of 21.8% and 21.5% for barium-impregnated and silver-lined catheters respectively, compared to control CSF that did not pass through a catheter. If the CSF was immediately sampled from the drain, the reduction was less, being 12.4% and 5.00% for barium-impregnated and silver-lined catheters respectively, however this reduction was still significant. T-tau levels were not significantly altered.

Adsorption of Aβ peptides to the luminal surface of lumbar drains needs accounting for when interpreting concentrations, to prevent misleading diagnosis or inaccurate results.

KEYWORDS: idiopathic normal pressure hydrocephalus (INPH); Alzheimer’s disease (AD), T-tau; AB1-42; cerebrospinal fluid (CSF); neurodegenerative markers
1. INTRODUCTION

The proteins Tau and Aβ1-42 are two conventionally measured cerebrospinal fluid (CSF) biomarkers that can assist with the diagnosis of neurodegenerative disease. In Alzheimer’s disease a high CSF total-tau (T-tau) concentration reflects neuroaxonal degeneration/injury and low CSF Aβ1-42 correlates with senile plaque pathology [1,2].

There is evidence that these markers could be useful in the diagnosis of NPH. In NPH, lumbar CSF levels of Tau and Aβ1-42 are typically low or low/normal (respectively), and could potentially discriminate from Alzheimer’s disease, in addition to being a putative prognostic marker for shunt responsiveness [3-6].

When measured in the context of investigating NPH, CSF is usually sampled from the lumbar drain, in-situ as part of the diagnostic protocol [7,8]. Increasingly lumbar drains are also being used to research rostrocaudal gradients of neurodegenerative markers, or within a study protocol method to obtain longitudinal biomarker results (to avoid multiple lumbar punctures) [9-11].

CSF transfer between collection tubes can reduce the overall concentration of Aβ1-42 by 25%, due to adsorption to the ionic surfaces [12]. It is unclear if the same effect is observed when CSF is sampled from lumbar drain.

We investigate the effect of both silver-lined (Silverline®, Spiegelberg, GMBH & Co.) and barium-impregnanted (EDM, Medtronic) lumbar catheters, vs. lumbar puncture on concentrations of Aβ1-42 and T-tau in CSF.

2. METHOD

Collection of CSF
CSF was collected from two anonymous individuals of unknown disease via lumbar puncture (LP). CSF sample collection and storage methods were all in accordance with the consensus guidelines for CSF biobanking [13].
**Lumbar drain testing**

CSF was thawed at 21°C and centrifuged for 5 minutes. A volume of 800uL was injected into (1) 2ml Sarsted aliquot (control), (2) barium-lined LD 80cm (3) silver-lined LD 80cm. CSF was collected through a LD over 3 different time periods: 30 seconds, 1 minute and 5 minutes (drain clamped before allowing CSF to drain). CSF was then collected into 2ml aliquots (Sarstedt, Numbrecht, Germany). Each test was repeated three times per tube type, and per time protocol.

**Electrochemiluminescent immunoassay analysis**

Samples underwent biochemical and enzyme-linked immunosorbent (ELISA) analysis to measure concentrations of T-tau (INNOTEST hTAU ELISA, Fujirebio, Ghent), Aβ-42 (INNOTEST β-amyloid (1-42), Fujirebio, Ghent). Total protein (TP) was measured as a control. A technician prospectively recorded levels of T-tau and Aβ-42 and was blinded to the tube type and dwell time.

Longitudinal stability in the measurements was ascertained using an elaborate programme of internal quality control (QC) samples. The laboratory also takes part in the Alzheimer’s Association external QC programme for CSF biomarkers. Intra- and inter-assay coefficients of variation were 12-15% for Aβ1-42 and 3-12% for T-tau.

**Statistical analysis**

Mean CSF concentrations (pg/ml) were corrected for TP. Measurement of uncertainly (MU) in T-tau and AB1-42 is 3-47 and 9-17 pg/ml respectively. ANOVA (Geisser-Greenhouse correction) determined significance.

**3. RESULTS**

A Percentage reduction in Aβ1-42 pg/ml for barium-impregnated catheters and silver-lined catheters are 9.3% (range 7.4-11.1%, taking MU corrections into account) and 20.5% (range 11.3-22.3) respectively, when CSF had dwelled in the catheter tube for 5 minutes (table 1). This reduction was significant in the silver-line tubing group. T-tau levels were not significantly altered.

| Table 1. Five min. CSF dwell time and neurogenertaive marker concentrations (sample 1) |
A significant percentage reduction in Aβ1-42 pg/ml was observed for both barium-impregnated catheters and silver-lined catheters, dropping by 21.8% (range 11.6-26.9%) and 21.5% (range 11.8-30.1%) respectively, when CSF had dwelled in the catheter tube for 1 minute (table 2). T-tau levels were not significantly altered.

Although the percentage reduction in Aβ peptide was less, there was still significant reduction in Aβ1-42 pg/ml for both barium-impregnated catheters and silver-lined catheters, dropping by 12.4% (range 8.68-16.1%) and 5.00% (0.97-8.67%) respectively, when CSF had been pulled straight through the lumbar catheter tube in over 30-50 seconds (table 3). T-tau levels were not significantly altered.

4. DISCUSSION

There are no publications demonstrating the clinical or research implications of analysing CSF samples for neurodegenerative proteins, when taken from a lumbar drain. We demonstrate that
CSF taken from lumbar drains can have artificially low Aβ1-42 concentrations (regardless of the drain being barium or silver lined).

CSF collection methods have been previously scrutinised regarding this issue. It has been found that aspiration of CSF (as opposed to drip collection) does not alter the levels of relevant proteins [14]. Furthermore, transfer at room temperature (and not on ice), and transfer times over 24 hours also do not significantly alter the levels of Tau and Aβ1-42 [15]. The use of manometers during collection can reduce Aβ1-38/40/42 levels, but ultimately does not alter the ratio's measured [16]. Aβ peptides are known to be ‘sticky’ and can be adsorbed to ionic surfaces of collection tubes, particularly during serial transfers [12].

With lumbar drain CSF concentrations of Aβ1-42 being reduced by up to 20% there is potential for misdiagnosis of Alzheimer’s disease. Furthermore, our findings have important implications for clinical research, where lumbar drains are thought to be a more patient friendly method of CSF collection when serial samples are needed. This adsorption of Aβ peptides to the luminal surface of lumbar drains needs accounting for when interpreting concentrations, to prevent misleading diagnosis or inaccurate results.

The effect Aβ1-42 peptides adsorption is reduced when CSF is pulled through immediately (5% reduction in barium lined lumbar drains). If a diagnosis of Alzheimer’s rests upon the results, additional testing with a lumbar puncture would be prudent. We advocate taking the adsorption effect into account when writing clinical or research protocols involving lumbar drains and analysis of Aβ peptide concentration results.

RESEARCH IN CONTEXT

1. **Systematic review**: There are no publications demonstrating the clinical or research implications of analysing CSF samples for neurodegenerative proteins, when taken from a lumbar drain.
2. **Interpretation**: We demonstrate that CSF taken from lumbar drains can have artificially low Aβ1-42 concentrations.
3. **Future directions**: Both clinical and research protocols analysing CSF Aβ1-42 concentrations from lumbar drains should acknowledge this potential effect

5. REFERENCES
