# Short communications

## Cerebrospinal fluid markers of central nervous system injury in decompression illness – a case-controlled pilot study

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**Abstract**

(Shahim P, Arnell P, Kvarnström A, Rosén A, Bremell D, Hagberg L, Blennow K, Zetterberg H. Cerebrospinal fluid markers of central nervous system injury in decompression illness – a case-controlled pilot study. *Diving and Hyperbaric Medicine*. 2015 December;45(4):240-243.)

**Introduction:** Decompression sickness (DCS) may cause a wide variety of symptoms, including central nervous system (CNS) manifestations. The main objective of this study was to examine whether DCS is associated with neuronal injury, and whether DCS could result in altered amyloid metabolism.

**Methods:** Seven, male divers with DCS and seven age-matched controls were included in the study. All the divers were treated by recompression but the controls did not receive hyperbaric oxygen. Cerebrospinal fluid (CSF) samples were collected 7−10 days after the diving injury and at three months follow-up. CSF biomarkers of neuronal injury, astroglial injury/activation, and a range of markers of amyloid β (Aβ) metabolism, as well as two proinflammatory interleukins, were analysed using immunochemical methods.

**Results:** There were no significant differences in the best-established CSF markers of neuronal injury, total tau (T-tau) and neurofilament light, between DCS patients and controls or between the two sampling time points. Also, there were no significant changes in the astroglial or amyloid (Aβ)-related markers between DCS patients and controls. However, the only diver with CNS symptoms had the highest levels of CSF T-tau, Aβ38, Aβ40 and Aβ42.

**Conclusion:** The results of our study speak against subclinical CNS injury or induction of inflammation or amyloid build-up in the brain among the six DCS patients without neurological symptoms. Further research, including on divers with CNS DCS, is justified.

**Key words**

Decompression sickness; central nervous system; injuries; inflammation; biomarkers

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### Introduction

Decompression sickness (DCS) is considered to be caused both by in situ bubble formation from dissolved inert gas, and arterial gas embolism, in which alveolar gas or venous gas emboli enter the arterial circulation via intra-cardiac or pulmonary right-to-left shunts. Manifestations can range from musculoskeletal and skin symptoms, to neurological symptoms and death. The standard treatment is recompression in a hyperbaric chamber following well-established protocols.[1]

Studies suggest that persistent foramen ovale (PFO) increases the risk of neurological symptoms and also asymptomatic ischaemic episodes in DCS by providing a means for arterial gas bubbles to directly reach the brain.[2]

Data are accumulating which indicate that brain hypoxia plays a role in the pathogenesis of Alzheimer's disease.[3] One of the hallmarks of Alzheimer's is the accumulation of amyloid β (Aβ) plaques.[4] In animal models of Alzheimer's, transient hypoperfusion results in an acute increase in Aβ by induction of the β-secretase metabolic pathway of amyloid precursor protein (APP), with formation of diffuse Aβ plaques.[5] In humans, increased Aβ expression has been reported in pyramidal neurons of the hippocampus in response to cerebral ischaemia.[6] A variety of biochemical markers have been used to investigate Alzheimer's.[7]

The main objective of this study was to examine in a group of divers presenting with DCS whether there were biochemical signs of brain injury, including in the absence of overt neurological symptoms. Further, we examined whether DCS could result in altered Aβ metabolism and neuroinflamation.

### Material and methods

Seven male divers (average age 32, range 24–44 years) were recruited from June 2009 to May 2013 upon admission for hyperbaric oxygen treatment for DCS. All seven received a US Navy Treatment Table 6. The study was approved by the Ethics Committee for Medical Research at the University of Gothenburg. Written informed consent was obtained from all participants. Cerebrospinal fluid (CSF) was collected by experienced anaesthesiologists using 20-g Sprotte needles 7−10 days after the decompression incident.[8] This is the optimal time to detect neurological injury in acute conditions such as stroke[9] and traumatic brain injury.[10] A second sample

**Table 1**
Cerebrospinal fluid biomarkers measured in this case-controlled study of divers with decompression sickness

| | |
|---|---|
| Total tau | T-tau |
| Neurofilament light | NFL |
| Visinin-like protein | VILIP-1 |
| Glial fibrillary acidic protein | GFAP |
| mammalian chitinase-like protein | YKL-40 |
| Amyloid β | Aβ38; Aβ40; Aβ42 |
| Amyloid precursor proteins | sAPP-α; sAPP-α |
| Interleukins | IL-6; IL-8 |

was taken at three months follow-up, by which time any biomarker abnormality should have normalized.[9]

Of the seven patients, six had peripheral DCS symptoms and one patient had central nervous system (CNS) symptoms. Two patients had a PFO on transoesophageal echocardiography (TEE), while the rest had normal TEE. For comparison, seven age- and sex-matched controls were included from a previously described group of neurologically healthy volunteers, collected at the same hospital using identical sampling and sample handling protocols.[11]

### BIOCHEMICAL PROCEDURES

All CSF samples were collected by lumbar puncture in the L3/L4 or L4/L5 interspace, centrifuged at 2,000 g at 4°C for 10 min, aliquoted and stored at −80°C pending batch analysis. Three sensitive CSF markers of neuronal injury (total tau, T-tau; neurofilament light, NFL;[12,13] visinin-like protein 1, VILIP-1[12]), two markers of astroglial injury/activation (glial fibrillary acidic protein, GFAP; YKL-40[12]), and a range of markers of amyloid β (Aβ) metabolism,[14] as well as two proinflammatory interleukins (IL-6 and IL-8)[15] were assayed using standard immunochemical methods (Table 1).

### STATISTICAL ANALYSIS

For the paired observations, the Wilcoxon signed rank test was used. For the group comparison of the biomarkers versus controls, the Mann-Whitney *U* test was used. All tests were two-sided and statistical significance was determined at $P < 0.05$. For multiple group comparison, the Kruskal-Wallis test with post-hoc Dunn's test was used. All statistical analyses were performed using GraphPad Prism 5.0 (GraphPad Inc., San Diego, CA).

## Results

### CLINICAL DATA

Of the seven divers, six had upper limb (mainly shoulder) joint pains and one presented with join pains, vertigo and paraesthesiae. Delay to recompression treatment varied from nine to 57 hours, though all but one diver presented within 24 h. Two divers, including the one with central nervous system (CNS) symptoms, had a PFO, identified by transoesophageal echocardiography (TEE), while the other five had a normal TEE. None of the participants reported post-lumbar puncture headache.

### BIOMARKERS OF NEURONAL AND ASTROGLIAL INJURY

There were no statistically significant differences in CSF concentrations of T-tau, NFL and VILIP-1 between the two groups or over time (Table 2). There were also no significant differences in the level of CSF GFAP and YKL-40 compared to controls or over time (Table 2).

The diver with a PFO and CNS symptoms had the highest levels of T-tau at admission and at the three-month follow-up (632 pg·ml$^{-1}$ and 726 pg·ml$^{-1}$ respectively), whereas the diver with a PFO but no CNS symptoms had normal biomarker levels.

### BIOMARKERS OF AMYLOID PATHOLOGY

There were no statistically significant differences in CSF concentrations of Aβ metabolism between the two groups or over time (Table 2). The diver with CNS symptoms had the highest CSF levels at admission and at the three-month follow-up of Aβ38 (3,330 pg·ml$^{-1}$ and 3,625 pg·ml$^{-1}$ respectively), Aβ40 (19,235 pg·ml$^{-1}$ and 21,547 pg·ml$^{-1}$ respectively) and Aβ42 (2,190 pg·ml$^{-1}$ and 2,414 pg·ml$^{-1}$ respectively).

### INFLAMMATORY BIOMARKERS

There were no significant differences in the levels of CSF IL-6 in DCS patients compared to controls (Table 2). However, the levels of CSF IL-8 were lower in DCS patients during admission and at follow-up as well as compared to controls, but these decreases were not statistically significant (Table 2). There were also no differences in either CSF IL-6 or IL-8 levels over time. There were no correlations between the levels of any of the biomarkers and the time to recompression therapy (data not shown).

## Discussion

Biomarkers of neurological injury in divers with DCS have not been reported previously. We were interested to see whether DCS affects Alzheimer's-related amyloid metabolism or alters biomarkers for neuraxonal damage, astroglial injury, and inflammation. The main finding from this pilot study was that there were no differences in well-established CSF markers of neuronal injury, T-tau and NFL, between divers with DCS and controls or over time post injury and recompression treatment. These markers are

**Table 2**

Cerebrospinal fluid biomarkers (means, 95% confidence intervals) in seven divers with decompression sickness and seven age-matched controls; there were no significant differences between the initial and 3-month values or at either time between the divers and the control group (see Table 1 for details of biomarkers)

| **Biomarker** (pg·mL$^{-1}$) | **7–10 days after DCS** | | **3-month follow-up** | | **Control group** | |
|---|---|---|---|---|---|---|
| T-tau | 321 | 175–466 | 377 | 99–554 | 270 | 212–327 |
| NFL | 334 | 235–434 | 1,270 | 1,009–3,550 | 360 | 266–454 |
| VILIP-1 | 203 | 12–394 | 232 | 49–415 | 72 | 34–110 |
| GFAP | 247 | 136–357 | 246 | 136–356 | 245 | 44–447 |
| YKL-40 | 58,682 | 44,730–72,635 | 74,169 | 46,063–102,275 | 74,022 | 49,965–98,079 |
| Aβ38 | 1,915 | 1,291–2,539 | 2,014 | 1,228–2,799 | 1,903 | 1,527–2,278 |
| Aβ40 | 12,771 | 8,549–16,993 | 13,753 | 8,629–18,123 | 13,753 | 11,349–16,158 |
| Aβ42 | 1,412 | 961–1,863 | 1,354 | 869–1,839 | 1,473 | 1,249–1,697 |
| sAPP-α | 962 | 769–1,156 | 1,016 | 808–1,224 | 988 | 694–1,282 |
| sAPP-β | 679 | 459–899 | 670 | 423–976 | 613 | 429–798 |
| IL-6 | 1.2 | 0.84–1.54 | 1.1 | 0.80–1.34 | 1.2 | 0.78–1.6 |
| IL-8 | 45 | 35–56 | 44 | 32–56 | 55 | 44–67 |

highly sensitive and specific for CNS injury and can also identify sub-concussive neuroaxonal injury following mild head trauma.[16,9]

Compared to controls, the divers had slightly elevated CSF levels of VILIP-1 at both time points. However, the lack of dynamic change in this marker speaks against the elevation being related to DCS, and it was not statistically significant.

Nor were we able to address whether PFO per se is a risk factor for aberrant CSF biomarkers for neuronal injury and Aβ metabolism. In the one study on the association of PFO with Alzheimer's disease and vascular dementia, the odds ratios did not differ significantly.[17]

One plausible reason for the reduced levels of IL-8 in the divers might be the fact that the pro-inflammatory cytokines were measured at seven days post recompression, whereas previous studies have shown that the levels of pro-inflammatory cytokines may peak at earlier time-points. Further, hyperbaric oxygen therapy may also modify the levels of pro-inflammatory cytokines.[18,19]

Seven days after the diving accident was chosen for sampling as this is optimal for neuronal injury and Aβ markers, but perhaps not for cytokines. CSF sampling at an earlier time point was not considered for ethical reasons as it is unclear if CSF sampling closer to a diving accident could influence cerebrovascular bubble formation.

The main limitation of this study is the small sample size. Nevertheless, the results speak against subclinical CNS injury or induction of inflammation or amyloid build-up in the brain of divers without symptoms and signs of CNS injury. With only one diver presenting with neurological symptoms, no conclusions can be drawn with regard to more serious injuries. We believe that these findings warrant further investigation in a larger cohort of divers, including those with CNS DCS.

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**Acknowledgements**

We thank David Pazooki, all study participants and the recompression chamber staff.

**Funding**

The study was supported by grants from the Swedish Research Council, Swedish State Support for Clinical Research (LUA-ALF), the Alzheimer Foundation, the Knut and Alice Wallenberg Foundation and the Torsten Söderberg Foundation.

**Conflict of interest**

The authors report no conflicts of interest. KB has served on Advisory Boards for Eli-Lilly, IBL International and Roche Diagnostics.

**Submitted:** 24 February 2015; revised 09 July and 15 October 2015
**Accepted:** 19 October 2015

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