Neuron-specific enolase and S100B protein levels in recreational scuba divers with neurological decompression sickness

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Abstract

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Introduction: Neuron-specific enolase (NSE) and S100B protein are brain-origin proteins commonly described to assess the presence and severity of neurological injury. To date, there are limited data examining the influence of scuba diving on these biomarkers, particularly when symptoms of decompression sickness (DCS) occur. The purpose of this controlled study was to determine whether these serum neurochemical markers could be used as 1) indicators of neurological DCS and 2) predictors of incomplete recovery.

Methods: Fifty-nine divers with neurological DCS and 37 asymptomatic divers admitted for inadequate decompression, serving as controls, were consecutively enrolled between 2010 and 2012. Blood samples were collected at initial presentation up to 6 hours after dive completion (controls) or onset of symptoms (DCS divers). Biomarkers were quantified in non-haemolysed samples only. Clinical outcome was assessed at 6 months post-injury.

Results: The two groups did not differ regarding the variables examined, except for the total dive time which was slightly shorter in the control group. NSE, but not S100B protein, was higher in the DCS group than in controls ($P < 0.0001$). An NSE level > 15.9 μg L^{-1} determined by ROC analysis predicted DCS development with a specificity of 100% (95% confidence interval (CI) 90 to 100) and a sensitivity of 24% (95% CI 14 to 36). There was a trend towards a higher likelihood of residual neurological deficits above this cut-off value ($P = 0.08$).

Conclusions: Early determination of NSE was found to be useful for the diagnosis of neurological DCS with a high specificity. However, its clinical applicability in decision making for determining treatment as well as its prognostic value remains to be established. Reliability of S100B protein was not demonstrated in the present study.

Key words

Decompression sickness, central nervous system, brain injury, proteins, severity, outcome, diving research

Introduction

Neurological decompression sickness (DCS) in scuba divers is a rare event with an incidence estimated between 0.02 and 0.03% per dive.[1] This disorder is the leading cause of morbidity with potential residual deficits of around 30% reported in the literature.[2,3] While there have been a number of clinical scoring systems devised for acute neurological DCS that have proved reliable for the prediction of incomplete recovery, little in the way of research into biological markers in humans has been conducted to test their value in diagnosing DCS and assessing prognosis.[4–6] Numerous studies have documented a variety of haematological and biochemical changes associated with decompression stress or the occurrence of DCS, but their utility as diagnostic tools has not yet been proven.[7–10] Particular attention has focused on the measurement of haematocrit, which has been noted to rise in severe cases of neurological DCS.[11] However, normal values have also been observed commonly in patients with a poor outcome, limiting the prognostic performance of this test in routine clinical use. Recent work also showed that elevated plasma D-dimer levels during the acute phase of neurological DCS was associated with the occurrence of sequelae at three months but the sensitivity of the test is still rather low.[12]

Neuron-specific enolase (NSE), a glycolytic enzyme predominantly localized in the cytoplasm of neurons and cells with neuro-endocrine differentiation, and S100B, a calcium-binding protein found in abundance in astroglial and Schwann cells, are commonly elevated during the acute phase of neurological damage after global cerebral ischaemia, stroke and traumatic brain injury.[13–15] The value of these neurochemical biomarkers in spinal cord injury is still unknown with very few investigations conducted in this field of study.[16]

To date, there are limited data examining the influence of scuba diving on these biomarkers, particularly when symptoms of DCS occur.[17–19] The purpose of this retrospective observational study in a large cohort of divers was to determine whether serum NSE and S100B protein levels could be used as 1) supplementary indicators to a clinical diagnosis of neurological DCS and 2) predictors of incomplete recovery.

Methods

The ethics committee of Saint Anne's Military Hospital approved the study, and all patients gave their informed consent. Between January 2010 and February 2012, 94 recreational divers with clinical signs of neurological DCS and 38 asymptomatic divers referred for inadequate decompression (i.e., fast ascent, omitted decompression

Figure 1
Flow diagram describing the selection of DCS divers

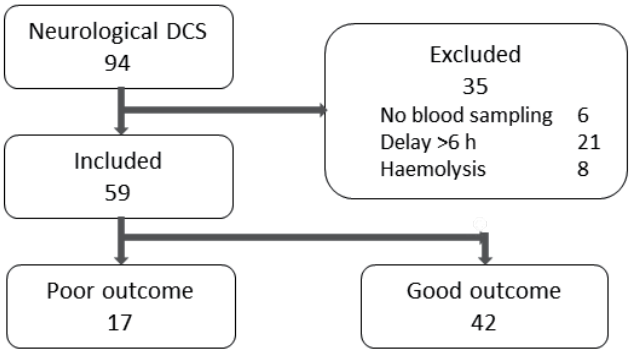


Table 1
Characteristics of DCS divers and control divers; mean (SD); * means $P < 0.05$

Characteristics	DCS divers	Controls	*P*-value
	n = 59	*n* = 37	
Age (years)	46 (10)	49 (12)	0.16
Gender (M/F)	43/16	27/10	0.82
Mean depth (msw)	40.5 (10.5)	41.5 (12.5)	0.65
Mean dive time (min)	35 (10)	30 (14)	0.02*
Repetitive dive	12/59	7/3	0.9
Delay for blood collection (min)	170 (70)	156 (34)	0.27

stops), serving as controls, were admitted to our hyperbaric facility (Toulon, France). Cases suspected of cerebral arterial gas embolism, patients with incomplete data or those who presented more than 6 hours after onset of symptoms (DCS divers) or more than 6 hours after surfacing (controls) were excluded. Demographics, diving parameters and delay between blood collection and dive completion were recorded in each group. Clinical outcome was classified as poor (presence of residual neurological manifestations defined as persistent objective sensory, motor or urinary disorders) or good (full recovery) after clinical evaluation at six months post injury.

Venous blood samples were collected from all divers on initial presentation and drawn in dry and EDTA tubes (8 ml). Serum NSE and S100B were obtained by centrifugation (5000 rpm for 10 min at 4°C) and stored at -80°C until measurement of both biomarkers with commercially available electrochemicoluminescence immunoassay kits (Elecsys, Roche Diagnostics). All samples with visible haemolysis were discarded from analysis to avoid any falsely elevated values for NSE.

STATISTICAL ANALYSIS

Data were expressed as mean ± SD or median with range for nonparametric variables. Differences between groups were compared using the unpaired Student's t-test or the Mann-Whitney U test where appropriate. Correlations between continuous variables were evaluated by calculating Spearman's coefficient (ρ). Associations between categorical variables were measured by the Fisher exact test. A receiver operating characteristics (ROC) curve was performed to discriminate the highest measurement of NSE levels in predicting DCS development while specificity (Sp) and sensitivity (Se) were obtained with the use of predefined thresholds. Odds ratios with 95% confidence intervals (CI) were calculated when needed and *P*-values lower than 0.05 were considered significant. Statistical calculations were performed with Graphpad Prism 5.00 (GraphPad Software, San Diego, CA).

Results

Fifty-nine DCS divers and 37 controls (after exclusion of 1 diver with haemolysis) were eligible for this study (Figure 1). Both groups were similar regarding the variables examined, except for total dive time which was shorter in the control group compared with DCS divers (35 ± 10 min vs. 30 ± 14 min, $P = 0.02$, Table 1). There was no significant difference in the mean delay to collection of blood between the groups (170 ± 70 min vs. 156 ± 34 min for DCS divers and controls, respectively).

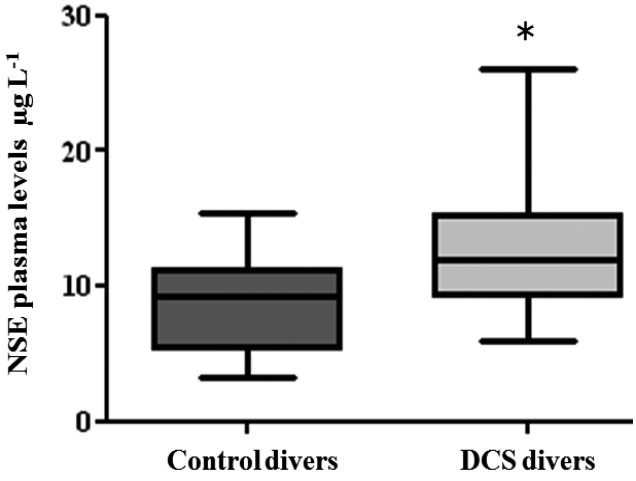
Of the 59 injured divers, 17 were found to have incomplete recovery after follow-up evaluation and were considered the severe group. Among them, four had disabling sequelae including urinary or bowel disturbance, ataxia due to sensory spinal myelopathy and mild degrees of limb spasticity. The remaining 42 (of 59) DCS divers did not exhibit neurological residual symptoms, and thus belonged to the benign group.

Serum NSE was higher in the DCS group than in controls (12.5 ± 4.3 µg L^{-1} vs. 8.8 ± 3.2 µg L^{-1}, $P < 0.0001$) (Figure 2). The level with the highest specificity and sensitivity was 12.1 µg L^{-1} (Sp = 89%, 95% CI 75 to 97; Se = 44%, 95% CI 32 to 58). A cut-off value of 15.9 µg L^{-1} predicted DCS development with a specificity of 100% (95% CI 90 to 100) and a sensitivity of 24% (95% CI 14 to 36).

The mean NSE level was significantly higher among patients in the severe group than those with a good outcome (14.5 ± 5.2 µg L^{-1} vs. 11.7 ± 3.6 µg L^{-1}; $P = 0.02$). However, association between NSE ≥ 15.9 µg L^{-1} and DCS severity did not reach statistical significance although there was a trend towards a poorer outcome above this cut-off value (OR = 3.5, 95% CI 0.99 to 2.3, $P = 0.08$).

There was no difference in the median S100B levels between injured divers and controls (0.087 ng L^{-1}, 95% CI 0.010 to 0.270 vs. 0.083 ng L^{-1}, 95% CI 0.045 to 0.260, respectively) or between severe DCS divers and those with

Figure 2
NSE concentrations in the serum of diver subgroups;
* $P < 0.0001$



benign evolution (0.081 ng L^{-1}, 95% CI 0.036 to 0.227 vs. 0.087 ng L^{-1}, 95% CI 0.010 to 0.272, respectively). In addition, there was no statistically significant correlation ($\rho = 0.08$) between NSE and S100B levels.

Discussion

To our knowledge, this is the first study investigating the concomitant use of NSE and S100B in divers with neurological DCS and comparing them to a control population. Our findings indicate that NSE, but not S100B, is elevated in the serum of divers presenting with neurological decompression sickness as compared to asymptomatic divers who had performed a dive with inadequate decompression. It appears that NSE is a specific biomarker which allows ruling in the diagnosis of neurological DCS with a very good reliability when the values exceed 12.1 µg L^{-1}. We also identified a cut-off value for NSE (> 15.9 µg L^{-1}) predicting the development of DCS with no false positives. However, the clinical usefulness of this test is hampered by its low sensitivity, meaning that a negative result does not necessarily rule out the occurrence of neurological DCS. In addition, the assay procedures make the clinical applicability difficult for the acute evaluation of the severity of DCS and consequently, for the choice of hyperbaric treatment regimen.

To our knowledge, there are only two reports assessing the influence of scuba diving on these two humoral indicators of neuronal damage.[17,18] Although no cumulative effect of repetitive dives on serum S100B levels was found in either study, there were small but significant post-dive increases in S100B concentrations in one study.[18] However, the concomitant rise in creatine kinase activity following each dive led the authors to suggest a skeletal muscle origin for this protein, as already observed after swimming.[19] In addition to S100B, NSE release did not seem be affected by four days of consecutive diving despite detection of significant amounts of vascular bubbles post dive.[18] These findings may indicate that uneventful no-decompression scuba dives do not cause discernable neuronal damage. On the other hand, a previous study in rats demonstrated a rise in serum S100B following simulated dives, with a strong correlation between S100B expression, bubble formation and/or the extent of hyperbaric exposure, suggesting a potential influence of decompression stress severity on alterations of the blood brain barrier.[20]

In a study of divers with neurological DCS, S100B also did not appear to be of clinical use in diagnosis as this marker did not increase over the next few days following the onset of symptoms.[21] Our data are in agreement with these findings, although the blood samples were drawn at different times, with an average time of less than 3 hours in the present study. To date, no clinical study has focused on the analysis of NSE concentration in DCS divers, hence making direct comparison with our findings difficult. Further research is warranted to evaluate the potential role of this biomarker in predicting outcome, in particular, after serial measurements over time since it has been reported that the release of NSE may reach a peak value at 48 to 72 h following acute ischaemic stroke.[22]

Conclusion

The present study reveals that plasma NSE concentrations in divers with neurological DCS exceed the levels found in control subjects who had performed dives with inadequate decompression. Our findings suggest that an increase of NSE level above a cut-off value of 15.9 µg L^{-1} measured early on admission appears to have a specificity of 100% but a low sensitivity for neurological DCS. The clinical relevance of this test in the acute assessment of divers with suspected neurological DCS remains to be established, considering the relatively long time needed to perform the biomarker analysis. The combined measurement of S100B with NSE does not add diagnostic or prognostic information, suggesting that damage of neurones is more involved in neurological DCS than glial alterations.

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Conflict of interest: None

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