Acute decompression illness and serum s100β levels: A prospective observational pilot study.

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Poff DJ, Wong R, Bulsara M. Acute decompression illness and serum s100β levels: A prospective observational pilot study. Undersea Hyperb Med 2007; 34(5):359-367. Background: S100β, a calcium binding protein associated with astroglial cells and other tissues has been shown to be raised in the serum of patients with a number of neurological pathologies. As there are no published data on serum S100β determinations in recreational divers affected by decompression illness (DCI) this pilot study determines whether S100β is a possible biochemical marker of DCI worthy of further investigation. Methods: Venous blood samples were drawn from patients diagnosed with, and treated for acute DCI at a hyperbaric facility and analysed for serum S100β concentration and Creatine Kinase (CK) activity. Samples were taken at initial presentation, and again following final treatment. Results: Twenty one patients were included in the study. Neither S100β, nor CK levels were significantly raised above population normal limits. Conclusion: S100β is not a clinically useful serum marker of acute DCI.

INTRODUCTION

Decompression Illness (DCI) refers to a disease state suffered as a consequence of bubble formation from dissolved inert gas within tissues or blood following a reduction in environmental pressure or by bubble introduction into the vasculature such as might be caused by pulmonary barotrauma. This term encompasses both Decompression Sickness (DCS) and Cerebral Arterial Gas Embolism (CAGE); the former referring specifically to a clinical or pathophysiological situation associated with bubble formation from dissolved inert gases, and the latter, to a situation of bubble gas introduction to the arterial blood (1).

The diagnosis of DCI is made purely on clinical grounds, involving the consideration of the history of events leading to the illness (including the dive profile(s), environmental conditions and post dive activity (such as airline flight), the symptoms complained of by the patient and the physical findings on examination of the patient. There are no laboratory tests that aid in the diagnosis or subsequent treatment of this condition, or indeed any that might serve to help a clinician to advise on issues including the period of time before a patient might safely return to diving or go to altitude (such as flying where cabin pressure is usually at 0.8ATA). Any such marker might be invaluable in both determining an appropriate treatment regime and assisting in prognostication.

Whilst the primary pathological
event in DCI is known to be the formation or introduction of bubbles into blood or tissues, the fundamental pathological process which gives rise to the clinical manifestations as diverse as, and including general malaise, joint ache, itch, soft tissue swelling, confusion, altered memory, paraesthesia or paralysis is not clearly understood. Tissue injury, including that to neurological tissues in the brain, spinal cord, and peripheral nerves, is thought to result in part from the mechanical disruption of blood supply to tissues and subsequently from the biochemical actions of a complex of inflammatory processes set in motion by this injury (2, 3).

Attempts to quantify the changes in physiological parameters in association with diving have led to the description of changes in hematological (4-7) and hormonal (8, 9) parameters. In addition, the relationship between elements of the coagulation and complement systems and diving, with or without the issue of DCI, has been investigated (10-12). Work on these aspects of diving physiology is ongoing and there is increasing interest in looking at aspects of proinflammatory mediators and markers of inflammation (13, 14). Much of this research has been focused on describing the underlying physiological change associated with diving in an effort to determine the basis of pathophysiological change resulting from DCI.

The S100 proteins are a subfamily of calcium binding proteins which have a diverse array of tissue associations and both intra- and extracellular functions (15). There has been a rapid evolution of the understanding of the numerous subtypes of these proteins, including S100β, an astroglial protein that is present in the blood of healthy persons in low levels at a concentration that is both age and sex independent (16). It has been associated with promise as a marker of brain tissue injury in both stroke and traumatic brain injury (17-19). Changes in levels of S100β have also been described in the settings of cardiac surgery both adult and paediatric as a potential indictor of neurological injury (20-22). It has been described as a possible surrogate marker for successful clot lysis in hyperacute cerebral artery occlusion (23) and as a pointer to the influence of peripheral neuropathy in Gullain-Barré syndrome (24). It has also been suggested that it may have value as a marker of both treatment and prognostic value in relation to carbon monoxide poisoned patients (25-27).

S100β is also regarded by some as a marker of physiological stress per se. Recent work indicates that this may be independent of the hypothalamic-pituitary-adrenal axis associated with glucocorticoid mediated stress responses (28). There is increasing understanding too, that S100β is also expressed in adipose tissue and skeletal muscle (29) and that it may not represent a marker with sufficient specificity to delineate neurological injury in for example acute head injury (30, 31). Further attempts to define S100β role as potential marker of neurological injury versus physiological stress have been carried out in a range of physical pursuits including marathon runners (32), elite ice hockey and basketball players (33), distance swimmers (34) and boxers (35). Data presented at the Breath Holding Diving Symposium in Orlando Florida, June 2006 by Andersson demonstrated increased serum levels of S100β in the 30 minutes following competitive breath-hold diving. The levels returned to baseline before 1 hour and again it is not clear if this brief increase in S100β level represents a normal stress response or is indicative of a pathological process. It is suggested that S100β has the role of a neurotrophic peptide in regular healthy exercise (36).

Efforts to differentiate the tissue source of S100β in situations of physiological stress versus possible neurological injury have included the use of correlation studies between
it and creatine kinase (CK)(32). Hasselblatt and colleagues demonstrated that CK determination may improve the specificity of S100β as a marker of brain tissue injury in the setting of acute trauma.

There is no published data on the levels of S100β in the diver, recreational or commercial, with symptoms of DCI. The aim of this study was to determine whether there is any significant rise in the serum levels of S100β in the diver clinically diagnosed with DCI either before or following treatment. In order to identify any confounding influence by an identifiable extraneural source of S100β, serum CK was measured concurrently.

MATERIAL AND METHODS

A total of 37 adult patients (> 18 years of age) presented to the Fremantle Hospital Diving and Hyperbaric Medicine Unit (FHDHMU) between 29 December 2004 and 13 February 2006. Of these, 11 patients were not enrolled because they either chose not to consent, or were not included due to failure on the part of the treating physician to consider the patient for enrolment. No patient was excluded on the basis of being too ill. The principal author was not involved in either the enrolment of subjects or the decision to treat.

The study group therefore consists of 26 consenting divers, all of whom were enrolled on the basis of the attending diving physician’s intention to treat with standard recompression therapy for the initial diagnosis of DCI. The diagnosis was made on the basis of clinical history and examination findings in the absence of alternative diagnosis in keeping with diving medicine practice. They were all recreational divers, 24 of whom were diving on SCUBA equipment and two were using surface supply “Hookah” equipment. Following enrolment into the study, five subjects were excluded from the analysis. One was excluded on the basis of an alternative diagnosis and was not treated for DCI. Four subjects who were enrolled were excluded from the final analysis having incomplete sets of blood results.

The study was given ethical consent by the South Metropolitan Health Services’ Human Research Ethics Committee and all subjects gave written consent for inclusion in the study and the storage of blood samples for the duration of the study.

Venous blood samples were taken prior to the subjects’ first recompression treatment, and again following their last. CK levels were assayed on a routine basis using a Cobas Integra 800 analyser (Roche Diagnostics, Castle Hill, NSW, Australia) with IFCC recommended reagents. As the S100β assay was a non-standard laboratory test, samples were stored at -20°C as recommended and batch analysed at intervals using an electrochemiluminescence immunoassay ‘ECLIA’ assay (Elecsys® 1010/2010/Modular Analytics E170, Roche Diagnostics GmbH, Mannheim, Germany). The measurement range for this assay is 0.005-39 μg/l.

Normal ranges for the two parameters were predefined by (i), the normal limits for serum CK activity used by the Fremantle Hospital Laboratory Services, and (ii), data provided by Roche Diagnostics as pertaining to the normal levels of serum S100β in healthy adults. This data accompanied the immunoassay kit.

Statistical analysis was done using both paired and single sample non-parametric tests; Wilcoxin signrank and Kolmogorov-Smirnov tests. Correlation between CK and S100β was examined using the method of Pearson. Mean ± sd are reported. The level of statistical significance was set at $p < 0.05$. All analysis was carried out using Stata (ref: StataCorp 2005. Stata Statistical Software: Release 9.
College Station, RX: StataCorp LP.)

No funding beyond the resources of the Fremantle Hospital Diving and Hyperbaric Medicine Unit was required for the completion of this study.

RESULTS

The average age of the 7 women and 14 men was 38 ± 8 years. The average time between the last dive undertaken and initial treatment was 3 ± 2 days and the average number of treatments was 3 ± 1. Patient demographics, symptoms and signs are detailed in Table 1, opposite page.

There was no rise in the serum concentration of S100β above the 95th percentile of the normal range in any of the divers diagnosed as suffering DCI prior to their initial treatment. The mean of the measured levels was significantly lower than the normal range 95th percentile (p<0.0001, single sample Kolmogorov-Smirnov test). There was also no discernable change in this parameter at the cessation of treatment, and there was no significant difference between the levels at the two time points. (p=0.639, Wilcoxin signrank test) (Figure 1)

Serum CK activity in the initial sample and the upper limit of the normal range were not significantly different (p=0.916, single sample Kolmogorov-Smirnov test). While there was a statistically significant difference between the initial mean CK activity and the final value (p=0.0007 Wilcoxin signrank test), both levels fell within the normal range of activity. (Figure 2)

There was no correlation between the CK activity levels and the measured serum S100β concentrations. (β-coefficient not significantly different to zero; p=0.663) (Figure 3)
Table 1. Subjects’ demographics, symptoms, and clinical signs as recorded by attending physician

<table>
<thead>
<tr>
<th>Subject</th>
<th>Gender</th>
<th>Age(years)</th>
<th>Symptoms</th>
<th>Signs</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>F</td>
<td>45</td>
<td>Nausea, unsteadiness, headache, altered facial sensation, fatigue, poor concentration</td>
<td>SRT&lt; 5 sec at best</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>39</td>
<td>Nausea, vomiting, fatigue, poor concentration</td>
<td>Inaccurate serial sevens</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>37</td>
<td>Intermittent head-ache, fatigue, poor balance, ache right elbow, axilla and finger tingling</td>
<td>16 sec SRT</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>38</td>
<td>Fatigue and lower back pain, itchy skin on back</td>
<td>Lower back paravertebral tenderness</td>
</tr>
<tr>
<td>7</td>
<td>F</td>
<td>35</td>
<td>Fatigue and right shoulder pain</td>
<td>NAD</td>
</tr>
<tr>
<td>8</td>
<td>M</td>
<td>50</td>
<td>Tender skin with rash on trunk,</td>
<td>Tender, blotchy rash; SRT&lt;30 sec</td>
</tr>
<tr>
<td>10</td>
<td>M</td>
<td>41</td>
<td>Headache, generalised myalgia, arthralgia (knees, ankles, elbows), paraesthesia in fingers and toes, poor concentration</td>
<td>SRT&lt;3 sec</td>
</tr>
<tr>
<td>11</td>
<td>M</td>
<td>52</td>
<td>Poor concentration, altered sensation in anterior thighs, arthralgia in knees, urinary retention</td>
<td>Decreased sensation over R L4/5, failed Romberg’s</td>
</tr>
<tr>
<td>13</td>
<td>M</td>
<td>53</td>
<td>Right shoulder arthralgia</td>
<td>NAD</td>
</tr>
<tr>
<td>14</td>
<td>F</td>
<td>28</td>
<td>L upper arm pain, swelling</td>
<td>L upper arm swelling (No DVT on U/S)</td>
</tr>
<tr>
<td>15</td>
<td>M</td>
<td>31</td>
<td>Chest pain, dyspnoea, polyarthralgia, general myalgia, severe fatigue</td>
<td>NAD</td>
</tr>
<tr>
<td>16</td>
<td>M</td>
<td>41</td>
<td>L. foot paraesthesia, paraesthesia right upper limb, upper trunk ache, neck pain, and clouded thinking</td>
<td>NAD</td>
</tr>
<tr>
<td>17</td>
<td>M</td>
<td>24</td>
<td>Generalised myalgia, fatigue headache, dizziness, transient paraesthesia in right arm and leg</td>
<td>Bilaterally grade I barotrauma, 40 sec SRT</td>
</tr>
<tr>
<td>19</td>
<td>F</td>
<td>48</td>
<td>Migratory polyarthrophy including elbow, lower back and knee, fatigue, mental clouding</td>
<td>NAD</td>
</tr>
<tr>
<td>20</td>
<td>M</td>
<td>33</td>
<td>Headache, migratory polyarthralgia</td>
<td>NAD</td>
</tr>
<tr>
<td>21</td>
<td>M</td>
<td>32</td>
<td>Progressive ache in Left shoulder and scapular, elbow, headache, nausea, poor concentration</td>
<td>Altered sensation to light touch on left shoulder, upper limb and hemiface, unsteady gait, inaccurate serial sevens</td>
</tr>
<tr>
<td>22</td>
<td>F</td>
<td>26</td>
<td>Dizziness, headache, transient left ankle pain</td>
<td>Anterior truncal rash with epigastric tenderness, &lt;10 sec SRT, Altered sensation L4 left leg</td>
</tr>
<tr>
<td>23</td>
<td>M</td>
<td>46</td>
<td>Scapular ache, R gluteal pain, Stiff neck, pain in right lateral arm, medial forearm, transient paraesthesia L hand</td>
<td>NAD</td>
</tr>
<tr>
<td>24</td>
<td>M</td>
<td>59</td>
<td>Altered sensation in both legs</td>
<td>Reduced power in R leg and altered sensation to light touch and vibration sense in R leg</td>
</tr>
<tr>
<td>25</td>
<td>M</td>
<td>21</td>
<td>Fatigue, ache and tingling in legs, SOB, chest tightness</td>
<td>NAD</td>
</tr>
<tr>
<td>26</td>
<td>M</td>
<td>33</td>
<td>Arthralgia left ankle, stiff left knee</td>
<td>Altered sensation to light touch on right foot</td>
</tr>
</tbody>
</table>

Notes:
1. SRT= Sharpened Romberg's Test; Technique - standing heal to toe, arms crossed over chest, eyes closed. Normal is balance held for minimum of 60 sec.
2. NAD= no abnormality detected
3. Subject 1, 5, 12, 18 excluded for incomplete data set, Subject 9 excluded for non-DCI diagnosis

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DISCUSSION

In the absence of any other reasonable explanation, the clinician presented with a recreational diver who has what is often a constellation of vague complaints, is left to conclude a diagnosis of decompression illness. This not infrequently occurs when the patient has no discernable abnormal clinical signs (Table 1), and indeed the dive profile(s) may not be regarded as provocative, that is, falls within accepted limits for depth and time. Regardless of the lack of palpable evidence, the treatment of the injured diver with recompression therapy brings satisfaction to both the patient and the clinician in the vast majority of cases. In this series of 21 divers, 70% had full resolution of their symptoms with treatment. Only 2 divers had a poor response, with the remaining having a partial relief of their symptoms.

There are a number of possible explanations as to why a patient with presumed DCI might not respond to therapy including delay to presentation and severity of illness. Frequently however, when the symptoms are vague, the clinician is left to wonder if the diagnosis truly is DCI and may elect to treat expectantly with standard recompression tables, interpreting a good clinical response as supporting the diagnosis of DCI. A quantitative marker of this pathological process could be helpful.

The use of biochemical markers to help diagnose and prognosticate permeates the practice of medicine in a number of disciplines. The usefulness of such markers is dependent on the positive predictive value of the test at the presumed prevalence of disease in the group of interest (the prior probability of disease). Routine application in the absence of an accurate estimate of prior probability may not be helpful for diagnosis. Hence they can on occasion be useful aids when used in a considered manner together with the clinical information.

Given that the understanding of the fundamentals of DCI pathophysiology is far from complete, it might be considered somewhat hopeful to pluck a new biochemical marker out of the literature and expect it to provide the desired outcome. The usefulness of S100β as a marker of neurological injury or physiological stress is debatable; however there is some rational with respect to diving injuries. The fact that other sports activities have been associated with an increase in its measurable activity both with and without the presumption of neurological injury raises the possibility of its applicability to the sport of diving. While recreational diving is not regarded as a typically strenuous activity when compared with marathon running, boxing or elite ice hockey it can be very physically stressful under certain environmental conditions such as adverse tide and wind. What is not known is how and why the apparent accumulated stress of tissue bubble accumulation gives rise to certain symptoms some of which have particular neurological features, such as parasthesia and disordered thinking or concentration.

This study recruited a typical cohort of patients that presented to a hyperbaric facility for assessment and treatment of apparently diving related illness. The decision to treat was undertaken by physicians experienced in diving medicine, and the treatment undertaken was routine. The outcomes of treatment as described by the treating physicians were equally typical with the majority of patients experiencing a full resolution of their symptoms. They were an ideal group to help determine whether or not S100β was worthy of further research into its usefulness as a biochemical marker of DCI.

There are a number of limitations of this study:

Not all of the 37 patients who were diagnosed and treated for DCI during the study period were recruited thus raising the issue
of selection bias. Whilst unlikely given the results, there remains a possibility that those recruited represent a subset of affected divers that were less likely to have a rise in serum S100β as a consequence of milder disease. This makes an assumption that those excluded were more severely affected and this cannot be corroborated.

There is no control population against which the subjects are compared. Whilst initially considered as part of the study design, it was felt that to attempt to find an adequately matched population for age, sex, dive profile and dive conditions would be beyond the scope of this initial pilot study. By the same token, to use an inadequately matched cohort, e.g. non-injured divers under a given set of conditions may not have provided an adequate solution. It was felt that in the event of results worthy of further investigation then these issues would need to be addressed by further study. Given the results this would appear unnecessary.

The diagnosis of DCI was made by experienced diving medicine physicians in keeping with their normal practice. As such the diagnoses were not made on the basis of explicit pre-study criteria with an assumption of diagnostic accuracy. There is therefore the possibility of selection bias and lack of external validity.

There was also an assumed pre-dive population normal value for each of the measured parameters. As there was no way to pre-dive test the affected patients, it is possible that their normal population values were different to those used. This does not seem likely as the normal values reported in a number of previous studies, with particular reference to those involving subjects undertaking other vigorous sporting activities, were similar to those used in this study. Raised levels of S100β in those studies were significantly above the baseline normal levels (32-35).

It is possible that a delay to presentation, diagnosis and treatment of on average 3 days would have influenced the levels of S100β since the half life of S100β has been shown to be about 30 min in patients after cardiac surgery (37). Previous studies of the relationship between S100β and a wide range of clinical situations as cited above have invariably been associated with the taking of samples in a period of less than 6 hours following the physiological insult under investigation. One could argue that despite ongoing symptoms in divers suffering DCI, the delay to investigation was time enough for any rise in S100β to be eliminated. One study did show that a single S100β sample taken at 48-96 hours post insult was indicative of successful clot lysis at < 6 hours in middle cerebral artery occlusion (23). However, given that the mean of the levels of S100β at both points of measurement were significantly lower than the population 95th percentile upper limit it can be concluded that the measured values were truly in the normal range. The sample size and the study design are not such that one might speculate that these low values represent other than normal range values. Given the desire to demonstrate clinical usefulness of S100β as a marker of disease in patients with DCI, this study’s results demonstrate real world applicability. A raised level of S100β theoretically discerned under controlled conditions of induced DCI, for example in an animal model, might have interest in terms of pathophysiology, but would not necessarily have any clinical relevance.

**CONCLUSION**

This pilot study supports the null hypothesis in that there is no significant change in serum levels of S100β in recreational divers suffering decompression illness at the time of treatment initiation. It provides data
that refutes any idea that the level of S100β could be clinically useful as a biochemical marker of decompression illness. It would also suggest, that if any of the symptoms attributed to decompression illness have their origin in underlying neurological injury, this injury will not be reflected in a change of serum S100β at the time of treatment.

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