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S100 AND COGNITIVE IMPAIRMENT AFTER MILD TRAUMATIC BRAIN INJURY

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Objective: **The aim of this study was to explore the relationship between the proteins S100B and S100A1B and symptoms and signs of cognitive impairment for 3 months after mild traumatic brain injury (MTBI).**

Methods: **Serum concentrations of S100A1B and S100B were examined in a prospective cohort study of patients with MTBI and a Glasgow Coma Scale score of 14 or 15. Cognitive performance was assessed by repeated computerized neuropsychological testing and an extended neuropsychological test. Symptoms were assessed using the Rivermead Post-Concussion Symptoms Questionnaire.**

Results: **Concentrations of S100B and S100A1B were above cut-off in 31% and 48% respectively. Eight percent of the patients had signs of cognitive impairment according to the computerized neuropsychological tests and 30% according to the extended test. Symptoms of cognitive impairment were reported by 44% of the patients on the first day post-injury and by 26% at 3 months. No significant associations between S100B or S100A1B concentrations and symptoms or signs of cognitive impairment were found.**

Conclusion: **Abnormal S100 serum concentrations and symptoms or signs of cognitive impairment were not significantly associated in patients with MTBI and a Glasgow Coma Scale score of 14 or 15.**

Key words: MTBI, biochemical markers, S100B, S100A1B, neuropsychology, cognition.

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INTRODUCTION

The severity of a traumatic brain injury (TBI) is usually defined by the patient's symptoms and signs at first presentation in the emergency ward and can be quantified by use of clinical rating scales, such as the commonly used Glasgow Coma Scale (GCS). Mild traumatic brain injury (MTBI) includes a history of altered or lost consciousness, post-traumatic amnesia and an emergency ward GCS score of 13–15 (1). However, it has been proposed that patients with a GCS score of 13 should be included in the moderate traumatic brain injury group due to similar risk of intracranial lesions and prognosis (2). The majority of patients with MTBI have a benign clinical course with symptoms such as headache and forgetfulness resolving within 3 months, but in a proportion symptoms persist longer (3). MTBI is also associated with signs of cognitive impairment such as problems of recall, speed of information processing and attention that in most cases resolve within 1–3 months after the injury (3). The pathophysiological basis for persistent symptoms and signs of cognitive impairment are far from clear. Several studies indicate that diffuse brain injury may also occur in MTBI, and that this might be below the detection threshold of the assessment methods routinely used, i.e. computerized tomography (CT) scanning or magnetic resonance imaging (MRI) scanning. Other methods to detect functional and structural disturbances might yield complementary information. These include biochemical markers of brain injury, such as the S100 protein. An association between serum concentration of the protein S100B and persisting subjective symptoms (4, 5) as well as signs of cognitive impairment (6) and disability (7) have been reported in traumatic brain injury of varying severity, but only a few of these studies have specifically addressed MTBI (4, 5).

Several S100 proteins (Soluble in 100% ammonium sulphate) have been described. In the nervous system 2 monomers predominate: S100A1 in neurones and S100B in all glial cells, as well as in a sub-population of neurones (8, 9). The monomer S100A1 and monomer S100B might form homodimers, S100A1A1 and S100BB, and the heterodimer S100A1B. Intracellular S100A1 and S100B proteins are involved in signal transduction, regulation of Ca^{2+} homeostasis and cell morphology. S100B also exerts extracellular functions and has been suggested to be involved in learning and memory (10), but little is known about the mechanism of secretion (9). Depending on its concentration, secreted S100B exerts trophic as well as toxic effects, and the effect in the context of traumatic head injury is unknown (11).

The specificity of S100B for TBI has been questioned (12, 13). A recent study of S100 proteins in patients with MTBI and patients with mild traumatic orthopaedic injuries suggested that S100A1B is more specific to MTBI than S100B (12).

Regarding the high incidence of MTBI (100–300/100,000 inhabitants) (14), a means of identifying patients with MTBI

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at risk of persisting complaints would probably enhance quality of care, by ensuring that patients needing intervention are adequately managed, and by avoiding unnecessary medical attention being paid to patients with MTBI with spontaneous recovery. Furthermore, in order to reduce long-term complaints and disability by preventive intervention or treatment, an understanding of the pathophysiological mechanisms involved is required.

The aim of the present study was to explore the relationship between S100B and S100A1B proteins and symptoms and signs of cognitive impairment for 3 months after MTBI in a prospective cohort study of surgically uncomplicated patients with MTBI, presenting with a GCS score of 14 or 15.

MATERIAL AND METHODS

Design

Patients with MTBI from 3 emergency departments (ED) were consecutively considered for entry in this prospective cohort study. The study was conducted between January 2000 and December 2001. Two of the EDs participated for a limited period: 6 and 15 months, respectively. Patients with high-energy traumas were admitted to a level-1-trauma centre in the region and thus were not available for the study. The total source population was about 800,000 inhabitants aged 15–65 years and living in the northern and central part of Stockholm County.

The local Ethics Committee approved the study and all subjects gave their informed consent.

Patients

Inclusion required all of the following: blunt trauma to the head with loss of consciousness (LOC) and/or amnesia, GCS score of 14–15 at the ED, injury within the past 24 hours, age 15–65 years.

Exclusion criteria were any of the following: amnesia >24 hours, LOC >30 minutes, no clear history of trauma as the primary event (e.g. epileptic seizures), other major injuries, major neurological disorders. Patients with previous or current psychiatric illness or alcohol dependence were not excluded.

A total of 122 patients were included, and neuropsychological data as well as S100 data were available in 97 patients (Table 1). Except for 2.3 years less education in the dropouts, no significant difference in any of the demographic or injury variables was found when comparing dropouts and non-dropouts.

Controls

In order to establish reference data, 35 non-injured controls were recruited by local advertisement. Inclusion criteria were: self-reported good health, age 15–65 years and no history of recent head trauma. Blood samples were collected and neuropsychological tests were assessed on 3 different occasions, at a first visit and 14 days and 3 months after the first visit. The mean age of the non-injured controls was 39 years. Forty-nine percent were male, and the mean length of education was 13.2 years. There were no significant differences between patients with MTBI and controls in age, gender distribution, education or occupation.

S100 analyses

S100 B was measured using a commercially available immunoluminometric assay (LIA-mat Sangtec S100 Sangtec Medical, Bromma. Sweden) described elsewhere (12). S100A1B was analysed using an enzyme-labelled immunosorbent assay (ELISA) method (CanAg Diagnostics AB, Gothenburg, Sweden) (12).

Pathologically elevated concentrations of S100 were defined as values above the 97.5 percentile in the non-injured control group (12). The cut-off level for S100A1B was 0.085 μg/l and for S100B 0.15 μg/l.

Table I. *Sociodemographic and clinical characteristics of patients with mild traumatic brain injury (MTBI)*

Characteristics	Patients with MTBI (n = 97)
Age (years)	
mean	37.2
range	15–65
Gender *n* (%)	
men	58 (60)
women	39 (40)
Years of education	
mean	12.45
range	3–19
Injury mechanism *n* (%)	
fall from height	18 (19)
fall	36 (37)
traffic (no car accidents)	0 (21)
assault	9 (9)
other	14 (14)
Affected by alcohol *n* (%)	23 (24)
GCS on arrival at hospital *n* (%)	
15	86 (89)
14	11 (11)
Injury-related pathological findings in CT and/or MRI	6 (6)
Loss of consciousness *n* (%) (minutes)	
0	26 (27)
0.1–0.9	20 (20)
1–5	35 (36)
6–30	16 (16)
Anterograde amnesia *n* (%) (minutes)	
0	2 (2)
0.1–0.9	15 (15)
1–5	21 (22)
6–45	36 (37)
>45	23 (23)

GCS = Glasgow Coma Scale; CT = computed tomography; MRI = magnetic resonance image.

Neuropsychological tests

Cognitive functions were assessed by repeated computerized neuropsychological testing as well as by an extended neuropsychological test.

Parallel versions of computerized tests from the Automated Psychological Test (APT) (15) were performed the day after the injury, after 14 days and after 3 months. The administered tests were: aspects of motor speed (Finger Tapping, F-test), focused and selective attention (K-test), reaction time (single and 2-choice visual reaction time and 2-choice with auditory inhibition, R-test) and long-term associative memory (O-test). The level of difficulty was adjusted to the subjects' actual performance by process control. A separate composite score was derived for each APT session based on 10 variables: 2 variables measuring motor speed, 1 measuring focused and selective attention, 4 measuring reaction time and 3 measuring long-term associative memory. As reference values, mean and standard deviation for each of the 10 test variables in the control group at each session were used. If patients performed 1 SD worse than mean for the controls in at least 2 separate (F-, K-, R-, or O-test) tests, at least 2 occasions, they were coded as having signs of cognitive impairment, possibly due to MTBI.

The extended neuropsychological test at 3 months post-injury included: Information, Digit Span and Digit Symbols from the WAIS-R (16), Block Span from the WAIS-R-NI (17), Buschke Selective Reminding Test (SRT) (18), The Stroop test (19), The Paced Auditory Serial Addition Test (PASAT) (20), The Trail Making Test (TMT) – parts A and B (18). Raw scores for neuropsychological tests at 3 months were transformed to standardized values according to the test manual.

The performance was considered abnormal if 2 or more test results were below 1 SD or if the results of separate tests differed by 2 SD or more.

An evaluation of cognitive function was then performed as follows. First, patients with abnormal cognitive function according to the criteria described above were identified. Secondly, in these patients pre-morbid factors that might be contributing to low cognitive performance were considered. Available data were age, years and type of education achieved and occupational history. Furthermore, in some cases information on level of degrees and anamnestic data concerning learning were available. Thirdly, when these factors did not explain the cognitive abnormality, current factors suggesting an alternative differential diagnosis were considered, such as body pain, other ongoing psychiatric or somatic disorders, sleep disturbance disability or history of possible brain injury. Patients were then classified into 2 groups: (i) patients with signs of cognitive impairment compatible with MTBI; and (ii) patients without such signs of cognitive impairment or with cognitive deficit compatible with causes other than MTBI. Two neuropsychologists independently rated all data with an interrater reliability of 0.85, $p < 0.001$. This classification was carried out with the computerized neuropsychological tests as well as with the extended neuropsychological test.

Reported cognitive symptoms

Symptoms were assessed by use of a Swedish version of the Rivermead Post-Concussion Symptoms Questionnaire (RPQ) (21) and cognitive symptoms (taking longer to think, poor concentration, poor memory) were specifically analysed. The RPQ score was calculated, as described by King et al. (21), as the sum of all symptom scores excluding ratings of 1, as these indicated that the symptoms caused by the trauma had resolved. Mild symptoms were scored as 2, moderate as 3 and severe as 4.

Procedures

The emergency ward staff documented GCS, amnesia, loss of consciousness, alcohol level according to a research protocol. Blood samples for S100 analyses were collected in the emergency department and the following day they were centrifuged; serum was separated and stored at −20°C until analysis.

All patients were examined by either computerized tomography (CT) scan within 24 hours post-injury and/or magnetic resonance imaging (MRI) within 7 days post-injury.

Computerized neuropsychological tests were performed the day after the injury, after 14 days and after 3 months (range 2.5–5.5 months) and an extended neuropsychological test was performed after 3 months.

Symptoms were assessed 1, 7, 14 days and 3 months (range 2.5–5.5 months) post-injury.

Statistics

All variables were summarized using standard statistics, such as mean, standard deviation, and frequencies. In this large sample, parametric methods were applied if the distribution of a variable was not severely skewed. In case of severely skewed distributions or outliers, the parametric methods were replaced by non-parametric methods. An α-level of 5% was used (2-tailed tests).

All S100 variables were dichotomized into normal or pathological levels based on the reference data from the control group. The classifications were made using the acute measurement. If the acute value was missing a conservative, non-biased method was used in order to increase the power of the analysis. Thus, the classification was then based on the value at day 1. If this value was missing too, the patient was withdrawn from the analysis.

For the analysis of the repeated computerized neuropsychological measurements, an ANOVA for repeated measurements was used (22).

Inter-rater reliability was calculated using the method of Bland and Altman (23). For the analyses of S100 variables and self-reported cognitive symptoms the Mann–Whitney test was used.

RESULTS

Thirty-one percent of the patients had S100B concentrations above cut-off and 48% had S100A1B concentrations above cut-off. A weak but significant and positive correlation was obtained between S100A1B and S100B (phi = 0.24, $p < 0.05$).

Eight percent of the patients had signs of cognitive impairment according to the repeated computerized neuropsychological testing and 30% of the patients had signs of cognitive impairment according to the extended neuropsychological test at 3 months post-injury. The relationship between the results of the 2 classifications of cognitive impairment was weak but highly significant (phi = 0.45, $p < 0.001$).

Forty-four percent of the patients reported 1 or more cognitive symptoms on the Rivermead Post-Concussion Symptoms Questionnaire on the first day, 45% on day 7, 27% on day 14 and 26% at 3 months.

There was no significant correlation between the dichotomized computerized neuropsychological test data and S100B ($\chi^2 = 0.14$, $p > 0.05$) or S100A1B ($\chi^2 = 0.30$, $p > 0.05$). Nor was there any significant relationship between signs of cognitive impairment according to the extended neuropsychological test at 3 months and levels of S100B ($\chi^2 = 1.61$, $p > 0.05$) or S100A1B ($\chi^2 = 0.30$, $p > 0.05$). This is illustrated in Fig. 1, which shows individual S100 data in relation to cognitive function.

Data from the computerized neuropsychological tests exhibited highly significant changes over time in several variables (i.e. better performance at 3 months), but self-reported cognitive symptoms were not related to neuropsychological test results. Separate analyses of the relationship between time development of the performance according to the computerized neuropsychological tests and pathological S100A1B or S100B did not reveal any significant interactions. Thus, there was no difference in the pattern of change over the session between patients with pathological S100 and those without. There was no difference in subjectively reported cognitive symptoms at any time point, between patients with and without S100A1B or S100B concentration above cut-off.

DISCUSSION

A substantial proportion of the patients with MTBI had S100A1B or S100B concentrations above the cut-off. Furthermore, a substantial proportion of the patients with MTBI was abnormal with respect to symptoms or signs of cognitive impairment, but these were not significantly correlated. We found no significant association between either symptoms or signs of cognitive impairment and the S100 protein abnormalities. Our data do not exclude a weak association but contra-indicate a clinically meaningful association (23) and do not favour S100A1B or S100B as a diagnostic tool to predict symptoms or signs of cognitive impairment after MTBI. This is in contrast to some other studies, where an association between the protein S100B and persistent symptoms (5), as well as signs of cognitive impairment (6) and disability (7), were reported in patients with traumatic brain injury of varying severity. However, only a few of these studies have specifically addressed MTBI (5, 24). Savola & Hillbom (5) reported that S100B predicts post-concussion symptoms. In that study, patients with a GCS score

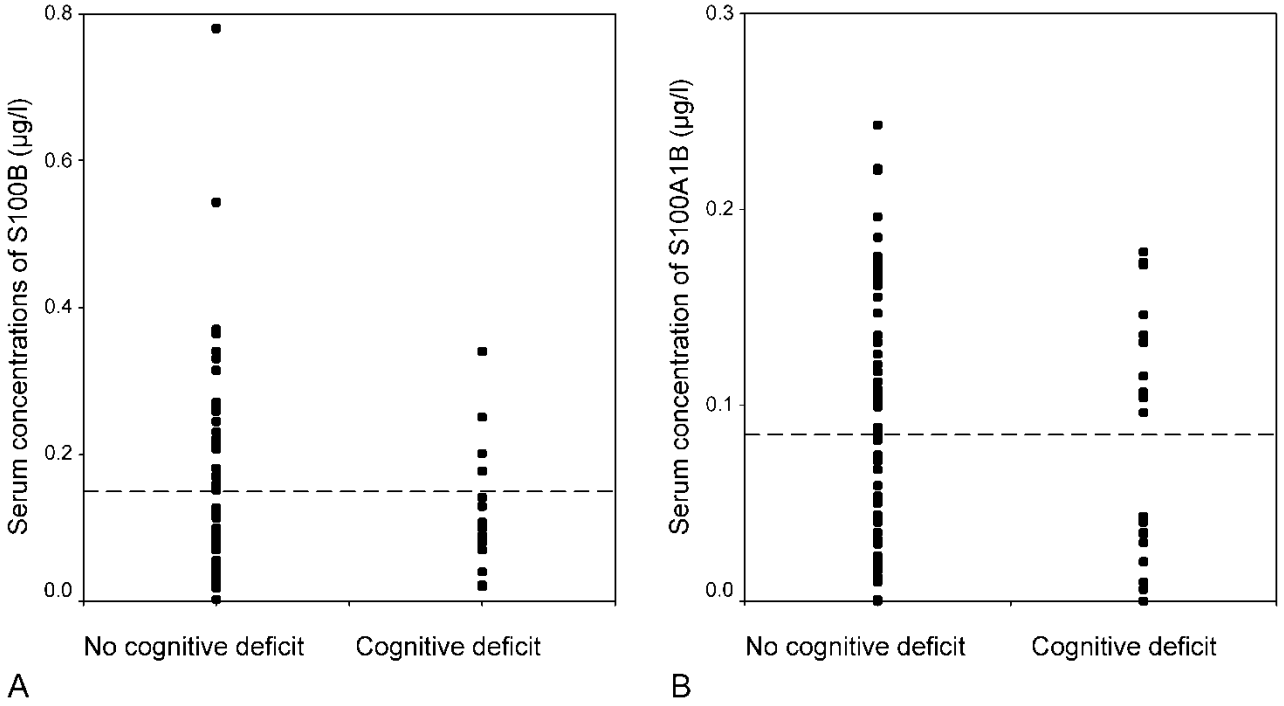

Fig 1. (A) S100B serum concentrations in patients with ($n = 25$) mild traumatic brain injury and without ($n = 71$) signs of cognitive impairment according to either the computerized neuropsychological test or the extended neuropsychological test at 3 months. Cut-off level at 97.5 percentile of non-injured controls, 0.15 µg/l (dashed line). (B). S100A1B serum concentrations in patients with mild traumatic brain injury with ($n = 25$) and without ($n = 72$) signs of cognitive impairment according to either the computerized neuropsychological test or the extended neuropsychological test at 3 months. Cut-off level at 97.5 percentile of non-injured controls, 0.085 µg/l (dashed line).

of 13 were included, and accordingly S100B concentrations were higher than in the present study. Furthermore, all symptoms collected by use of a modified version of the RPQ were considered while we specifically addressed cognitive symptoms according to the original version of that questionnaire. A recent study by Herrmann et al. (6) indicated that the acute S100B concentration predicts long-term neuropsychological impairment after MTBI. In that study, radiological abnormalities, according to CT scan or MRI scan, were present in 48% of the patients. The frequency of radiological abnormalities in the current study was only 6%, which is similar to that reported in CT scan studies of unselected patients with MTBI with a GCS score of 15 (25, 26). Thus, the present cohort study included patients within the milder MTBI spectrum, i.e. mainly with a low-energy injury mechanism and a GCS score of 14 or 15 on arrival at the ED, and probably representative of the majority of patients with MTBI.

The interpretation of these findings must consider limitations of the assessment methods as well as the unknown pathophysiological basis for persisting symptoms or signs of cognitive impairment in these patients with MTBI. Several previous studies have shown abnormal elevations of S100 proteins in patients with MTBI (4, 27, 28) as was also observed in the present study. The specificity of these elevations for traumatic impact on brain tissue has been questioned (13). Recently, we observed that S100A1B might be more specific to brain injury than S100B, when S100 levels in patients within the upper MTBI spectrum and patients with mild orthopaedic injuries but with no head injury were compared (12). However, in the current study, none of the S100 elevations was associated with cognitive impairment and thus these cannot be used to link the observed cognitive impairment to an organic, pathophysiological mechanism. It might be pointed out that the origin, release mechanism and function of the different S100 proteins are still poorly characterized and that S100B is suggested to be either potentially detrimental or beneficial after a brain lesion (11). It has also been suggested that up to a certain level, increase in S100B reflects a disturbed blood brain barrier due to energy transfer to the head, and not necessarily an impact on the brain (29), and that more intense injury is required to make S100B reflect an impact on brain tissue. A better understanding of the pathophysiological mechanisms behind the observed S100 elevations is necessary. One way to increase understanding in this respect would be to compare S100 data with the results of other measures of brain injury structure and function, such as new imaging techniques and other biochemical markers, e.g. glial fibrillary acidic protein (GFAP).

Several previous studies have shown impaired cognitive function that mainly resolves within 3 months after MTBI (3, 30, 31). In the present study a substantial proportion of the patients with MTBI exhibited signs of cognitive impairment after 3 months. The classification of cognitive impairment as

compatible with MTBI or not was developed in order to minimize the influence of possible confounding factors such as depression, and risk factors such as pain. Remaining effects of such factors cannot be fully ruled out and thus might obscure an association between the S100 proteins and signs of cognitive impairment. The lack of a significant association between the S100 proteins and cognitive symptoms might also be related to the unspecific character of these symptoms. Several studies have pointed out that symptoms reported after MTBI are not unique, even if they are more common within the first month after an MTBI than after other injuries or in the general population (30, 32, 33).

In conclusion, this study showed no significant association between abnormal S100 proteins in serum and symptoms or signs of cognitive impairment in patients with MTBI presenting with a GCS score of 14 or 15.

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