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Journal of the Neurological Sciences 240 (2006) 85 – 91

Journal of the Neurological Sciences

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Increased serum-GFAP in patients with severe traumatic brain injury is related to outcome

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Received 18 March 2005; received in revised form 13 September 2005; accepted 13 September 2005
Available online 2 November 2005

Abstract

Objectives: Several studies have established the relevance of S-100 in blood as a marker of brain damage after traumatic brain injury. However, a more specific marker is required and glial fibrillary acidic protein (GFAP) is considered to be a good candidate.
Methods: In order to assess the increase of GFAP in serum (s-GFAP) after a severe traumatic brain injury (TBI) we collected daily serum samples from 59 patients with severe TBI starting on the day of the trauma. S-GFAP was measured using a sandwich ELISA. The Glasgow outcome scale (GOS) assessed outcome after 1 year.
Results: All but one patient had maximal s-GFAP values above the laboratory reference value (median increased 10-fold). The highest detected levels were seen during the first days after TBI and then decreased gradually. Patients with unfavourable outcome had significantly ($p<0.001$) higher maximal s-GFAP values in the acute phase compared with patients with favourable outcome. All patients ($n=5$) with s-GFAP >15.04 μg /L died (reference level <0.15 μg/L). We found no significant difference in the maximal s-GFAP levels of patients with isolated brain injury in comparison with patients with multiple traumas.
Conclusion: Serum-GFAP is increased during the first days after a severe traumatic brain injury and related to clinical outcome.

Keywords: Glial fibrillary acidic protein (GFAP); Serum; Traumatic brain injury; Outcome; GOS; Biochemical brain markers

1. Introduction

Traumatic brain injury is a leading cause of death and disability in children and young adults in developed countries. “The Lund concept”, which advocates aggressive antioedema treatment of high intracranial pressure at moderate central perfusion pressure (CPP), has been reported to improve outcome [3,4]. Despite significant progress in cerebral monitoring it is still difficult to quantify the extent of the primary brain injury and ongoing secondary damage. Because of the limitations of clinical and radiological assessment there has been considerable interest in developing biochemical methods both to measure the extent of brain damage and to improve outcome prediction.

S-100 β is a protein of astroglial origin, which has been used as a serum marker of central nervous system (CNS) damage the last decade. An association between clinical outcome and serum concentrations of S-100 β in patients with severe TBI has been shown [5]. However, S-100 is expressed not only in brain tissue but also in a variety of other cell types in both physiological and pathological conditions. Expression of the S-100 β protein has been observed in fat, skin and skeletal muscle [6].

In contrast GFAP is found only in the nervous system. GFAP is a structural protein of the intermediate filament of astroglia. Significantly increased levels of GFAP are observed in cerebrospinal fluid (CSF) as a consequence of acute CNS injury and modestly increased levels of GFAP

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doi:10.1016/j.jns.2005.09.007

are seen in patients with astrogliosis [7]. Numerous reports document the usefulness of CSF–GFAP as an indicator of CNS pathology [7–9]. After brain damage proteins like GFAP are released from injured brain cells and appear in the systemic circulation probably directly via passage through a disturbed blood brain barrier. Serum analysis has clinical advantages over CSF measurement and CSF sampling is often contraindicated in patients with severe traumatic brain injury. Missler et al. reported a method for serum-GFAP determinations and preliminary results from patients with severe head trauma in 1999 [1]. They concluded that measurement of GFAP concentrations in blood appeared to have the possibility to identify acute CNS damage. Vos et al. [10] have recently presented a study showing that serum levels of glial and neuronal proteins in the very early phase after TBI predict outcome. A few months later Pelinka et al. [11] confirmed that GFAP is related to outcome after TBI and they also concluded that GFAP is not released after multiple traumas without brain injury.

The aim of this prospective study was to evaluate consecutively measurements of serum-GFAP in the acute phase of severe TBI, and to investigate if serum levels are related to outcome after 1 year.

2. Material and methods

All patients with severe TBI admitted to the Neuro-intensive Care Unit (NICU) at Sahlgrenska University Hospital between October 2000 and December 2002 were consecutively included in this prospective study. The trauma was defined as severe if the following criteria were all fulfilled:

1) Reaction Level Scale (RLS) $\geq$4, corresponding to a score sum of $\leq$8 on the Glasgow Coma Scale [12].
2) A therapeutic indication to monitor intracranial pressure (ICP).
3) Need for ventilator treatment.

Some patients were transferred to NICU from other intensive care units, but to be included in the study the first blood sample had to be obtained on day 2 at the latest. The day of the trauma was defined as day 0. Venous blood samples for GFAP were obtained as soon as possible after admission to NICU and then every morning on day number 1, 2, 3, 4, 6, 8 and once in the period between days 11 and 14.

Since we aimed to follow the patients until one year after the trauma only patients living in Sweden were included in the study. The Ethics Committee at the University of Göteborg approved the study. The closest relative gave informed consent. All patients were treated according to a standardised protocol, "the Lund concept" aimed at maintaining a cerebral perfusion pressure of >60 mm Hg and an intracranial pressure of <20 mm Hg [4,13]. The concept is based on physiological principles for volume regulation of the intracranial compartment. Main components are normovolemia, normotension and reduction of cerebral metabolism and stress response. Gradually increased medical treatment can be combined with neurosurgery.

Vital signs and ICP were monitored on an hourly basis. Indication for neurosurgery was clinical and individual. One neuroradiologist (I. N.) reviewed all initial CT according to Marshall categories I–IV. Since she was blinded to clinical and laboratory data, mass lesions were also classified due to their consequences on midline shift and compression of cisterns, and not according to a retrospective analysis of evacuation or not [14]. Thus the CT grading was modified into IV types. After 12 months s-GFAP was reassessed. Outcome was measured using the Glasgow outcome scale (GOS). We used a structured questionnaire based interview [15]. The examiner was blinded to the s-GFAP data.

Serum-GFAP was measured using a modified sandwich ELISA as described by Rosengren et al. [7]. In short the assays were run in microtest plates using hen anti-GFAP IgG as the capturing antibody. Duplicate samples of serum (50 μL) were incubated with phosphate buffered saline (50 μL) in each well. Duplicate samples of reference GFAP (0.062–8 μg/L) were incubated in phosphate buffered saline supplemented with 50% normal horse serum (Sigma, USA). Rabbit anti-GFAP IgG was used as the detection antibody. Bound rabbit IgG was detected by the binding of peroxidase-conjugated donkey antirabbit IgG. The colour reaction was developed using ï-phenylenediamine and Perhydrol and the optical density was measured at 490 nm. The concentrations of GFAP were interpolated from the standard curve. Interassay precision was determined by duplicate analyses of two CSF samples and one serum sample at 71 different days. Mean intraassay precision was determined using the same samples run in 4 duplicates at 14 different days. Linearity of the assay was controlled by serial dilutions of three patient samples with very high levels of GFAP in phosphate buffered saline supplemented with 50% normal horse serum. Recovery of the assays was determined by spiking serum from 14 normal controls with reference GFAP at 2.0, 1.0 and 0.50 μg/L. To determine GFAP reference levels serum samples from 218 healthy individuals (mean age 46.0 years, range 18–80) were analysed.

In general, statistical analysis was performed using non-parametric tests because the data didn't follow a Gaussian distribution. For comparison between two groups Mann–Whitney *U*-test was used for continuous variables and for dichotomous variables Fisher's exact test was used. In order to test ordered categorical variables between two groups Mantel Haenszel's test was performed. Pair wise Mann–Whitney tests (Van Elterens test) were used to test differences between groups, adjusting for neurosurgery. All variables significantly ($p<0.10$) correlated to dependent variables were entered in a forward stepwise multiple logistic regression. In that analysis s-GFAP was log trans-