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Comparison of Serial S-100 and NSE Serum Measurements after Severe Head Injury

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Summary

We investigated the time course of neuron specific enolase (NSE) and S-100 protein after severe head injury in correlation to outcome. We included 30 patients (GCS < 9), who had been admitted within 5 hours after injury, in a prospective study. Blood samples were taken on admission, 6, 12, and 24 hours and every 24 hours up to the fifth day after injury. The outcome was estimated on discharge using the Glasgow Outcome Scale. 70% reached a good outcome. All concentrations of NSE and 83% of the S-100 samples were elevated concerning the first probe (30.2 µg/l NSE mean and 2.6 µg/l S-100 mean). Patients with bad outcome had an NSE concentration of 38 µg/l (mean) compared with 26.9 µg/l (mean) in patients with good outcome. Patients with bad outcome had an S-100 concentration of 4.9 µg/l (mean) compared with 1.7 µg/l (mean) in patients with good outcome ($p < 0.05$). The mean values of NSE and S-100 decreased during the first 5 days. Four patients with increasing intracranial pressure showed a quick increasing concentration of NSE, in two patients the S-100 level showed a slower rise. The NSE serum levels did not correlate with intracranial pressure values. Our results show that the first serum concentration of S-100 seems to be predictive for outcome after severe head injury.

Keywords: Head injury; S-100 protein; NSE; outcome.

Introduction

Neuron specific enolase (NSE) and S-100 protein are known indicators of brain damage [1, 6, 8, 9, 11–15, 17, 18]. The NSE is a glycolytic enzyme of the cytoplasm of neurons and the cells of the APUD system [1, 5]. The S-100 is a calcium binding protein, which is localized in astroglial cells in the central nervous system [3, 5]. Skogseid found in his population, comparing patients with severe and moderate to minor head injuries, a correlation of NSE serum level with clinically assessed severity of injury and with contusion volume estimated from computerized tomography, respectively [15]. A recently published case report showed a correlation between increased S-100 serum levels and structural brain damage detected by MR after minor head injury [6]. Skogseid and Ingebrigsten recommended further studies to evaluate the NSE and S-100 serum levels in relation to outcome [6, 15]. As far as we know there is still no study which investigates the time course of NSE and S-100 serum levels after severe head injury correlating these to outcome. The aim of this study was to provide further information in this field.

Method and Patients

After obtaining the approval of the local ethics committee, we included 30 patients with severe head injury (Glasgow Coma Scale [16], GCS < 9, Table 1), who had been admitted within 5 hours after injury, in a prospective study. They consisted of 23 males and 7 females. The mean age was 32 years with a range from 17 to 73 years. We excluded patients after resuscitation or shock and with a known neurological disease or spinal cord injury. Seventeen patients had an isolated head injury, ten patients had a concomitant injury of the thorax and/or abdomen. Three patients had additional fractures of the extremities. Twelve patients had been operated on for an intracranial mass lesion and/or depressed skull fractures, another 5 for abdominal bleeding and 3 had been operated on for skeletal fractures.

All patients received a cranial computerized tomography (CT) on admission, the findings had been classified according to the CT classification of The Traumatic Coma Data Bank, see Table 2 [10].

Blood samples were taken on admission (mean 2.5 hours), 6, 12, and 24 hours after trauma and every 24 hours up to the fifth day after injury. After separation, the serum was stored at –20 °C until analysed for NSE and S-100 concentrations by using a commercial ELISA kit (Wallac®) and an RIA (Byk-Sangtec®). Samples were analysed in duplicate, the mean value of these samples was taken into account for further evaluations. The coefficient of variation between the single samples is for NSE 9.3% mean (min. 0.1%; max. 34%) and for S-100 5% mean (min. 0%; max. 32%). NSE

concentration of 10 µg/l or above and S-100 concentrations of 0.5 µg/l or above were defined as being increased.

In addition, we registered the intracranial pressure (ICP) in 18 patients at the time when blood samples were taken. The outcome was estimated on discharge (mean 19 days after trauma) using the Glasgow Outcome Scale (GOS 1–2 = bad, GOS 3–5 = good) [7].

Statistical analysis was performed with the computer programme StatView 4.5 ®. Data analysis included the Fisher's PLSD, the Scheffé's and the Bonferroni/Dunnett's post hoc tests to evaluate differences between the outcome groups. Regression analysis of the levels of NSE, S-100 and ICP was carried out. The statistical significance was determined at a level of $p < 0.05$.

Table 1. *Glasgow Coma Scale on Admission Correlated with Outcome*

Glasgow coma score on admission	Count	Per cent	Outcome[a], count	
			Good	Bad
3	11	36%	5	6
4	3	10%	3	0
5	2	7%	2	0
6	6	20%	5	1
7	6	20%	5	1
8	2	7%	1	1

[a] According to Glasgow Outcome Scale (GOS), 1–2 = bad, 3–5 = good.

Results

Outcome

70% of the patients reached a good outcome according to the Glasgow Outcome Scale at discharge. 23% died during the first 5 days and 7% remained vegetative after injury (Table 2). The initial CT findings correlated with outcome ($p < 0.05$, Table 2). 66% of the patients with bad outcome had an initial CT scan category between 4 and 6, of the patients with good outcome only 20% had a CT scan in these categories. The initial GCS did not correlate significantly with outcome, despite this there seems to be a strong trend (Table 1). Six of nine patients with bad outcome had an initial GCS of 3. Age did not show any correlation with outcome.

Serum Levels of NSE and S-100 Protein

All concentrations of NSE were elevated concerning the first sample. The mean values of NSE decreased during the first 5 days from 30.2 µg/l to 14.4 µg/l. Patients with bad outcome had an NSE concentration of 38 µg/l (mean) concerning the first sample compared with 26.9 µg/l (mean) in patients with good outcome. This difference failed to be statis-

Table 2. *Diagnostic Categories of Types of Abnormalities Visualized on CT[a] Scanning Correlated to Outcome*

Category of CT findings	Definition	Count	Per cent	Outcome[b], count	
				Good	Bad
Diffuse injury I	No visible intracranial pathology seen on CT scan	3	10%	3	0
Diffuse injury II	cisterns are present with midline shift 0–5 mm and/or: lesion density present no high- or mixed-density lesion > 25 cc may include bone fragments and foreign bodies	13	43%	12	1
Diffuse injury III	cisterns compressed or absent with midline shift 0–5 mm, no high- or mixed-density lesion > 25 cc	4	13%	2	2
Diffuse injury IV	midline shift > 5 mm, no high- or mixed-density lesion > 25 cc	2	7%	0	2
Evacuated mass lesion	any lesion surgical evacuated	0	0%	0	0
Non-evacuated mass lesion	high- or mixed-density lesion > 25 cc, not surgically evacuated	8	27%	4	4
Sum		30	100%	21	9

[a] CT computed tomography.

[b] According to the Glasgow Outcome Scale (GOS): 1–2 = bad, 3–5 = good.

Table 3. *Time Course of Serum Levels of NSE and S-100 After Severe Head Injury*

Time after injury, hours		Sample 1 2.50 (mean)	Sample 2 6	Sample 3 12	Sample 4 24	Sample 5 48	Sample 6 72	Sample 7 96	Sample 8 120	Outcome[c]	
										Good sample 1	Bad sample 1
NSE	Mean, µg/l	30.2	23.6	16.6	16	15.9	9.4	12.5	14.4	26.9	38[a]
	Min.	10.4	6.4	3.5	2.58	0.1	0.1	0.1	0.1	10.4	13.6
	Max.	103.4	60.4	56	64.8	112.4	24.8	47.7	63.3	103.4	84.7
	Std. deviation	22.9	14.4	12.8	14.9	21.8	7.1	11.5	15	21.7	25
S-100	Mean, µg/l	2.6	1.3	1.1	1.3	1.3	0.6	0.5	0.4	1.6	4.9[b]
	Min.	0.35	0.2	0.23	0.2	0.25	0.23	0.23	0.21	0.35	0.7
	Max.	16.2	8.8	9.1	18	14.5	4.7	2.8	3	10	16.2
	Std. deviation	3.6	1.7	1.7	3.4	2.8	0.9	0.6	0.6	2	5.3
Number of patients		30	28	28	28	28	26	23	23	30	30

[a] Non significant; [b] $p < 0.05$ according to Fisher's PLSD, Scheffé's and Bonferroni/Dunnett's post hoc tests.
[c] According to the Glasgow Outcome Scale; bad = 1–2, good = 3–5.
Normal value for NSE < 10 µg/l and S-100 < 0.5 µg/l.

tically significant. For further details, see also Table 3.

Four patients showed an increasing NSE serum level which correlates with an increasing intracranial pressure. Three of them died due to untreatable brain oedema and one patient had been operated on for a contusion with a favourable outcome (GOS 3) and postoperatively low NSE serum values.

Eighty-three per cent (n = 25) of the S-100 concentrations were elevated concerning the first samples taken. The mean values of S-100 decreased during the first 5 days from 2.6 µg/l to 0.4 µg/l. Patients with bad outcome had an S-100 concentration of 4.9 µg/l (mean) concerning the first samples compared with 1.7 µg/l (mean) in patients with good outcome ($p < 0.05$). Of the four patients, mentioned above, with increasing ICP, only two showed a significant increase of S-100 serum concentrations during increasing ICP levels.

We correlated all ICP values (n = 94) with the serum NSE and S-100 concentrations and found no significant correlation concerning the serum levels with ICP (NSE–ICP: $R^2 = 0.33$; S-100–ICP: $R^2 = 0.56$). Also the NSE values did not correlate with the S-100 values ($R^2 = 0.25$). The initial serum levels of S-100 and NSE did not correlate with the GCS on admission neither with the CT classification of the Traumatic Coma Data Bak.

Discussion

We show in our prospective study that early serum levels (< 5 hours) of NSE and especially of S-100 after severe head injury seem to be predictive concerning the outcome.

In contrast to brain damage after focal ischaemia, the early concentration peak of these markers reflects the mechanical disruption of brain tissue [3]. Hardemark saw in his experimental cortical contusion studies on rats an early increase of NSE and S-100 within 7.5 hours after impact [3]. The concentration declined immediately and increased after 1.5 days, suggesting secondary brain damage [3]. We can confirm these observations in patients with severe head injury. Within the first 12 hours after trauma the highest values of NSE and S-100 were found in the first samples taken after 2.5 hours (mean) following injury (Table 3). Further increasing values of these markers especially of NSE correlated with increasing ICP, reflecting secondary brain damage. Interestingly the serum levels of NSE did not correlate with S-100 concentrations, suggesting that the markers have a different sensitivity. In contrast to S-100, which seems to be a significant marker of primary brain injury, the NSE serum levels seem to be a potential marker, which indicate secondary brain damage. In four patients with rising ICP the serum levels of NSE increased before the absolute values of the ICP reached life-threatening levels. The S-100 serum levels did not

show a prompt increase of concentrations. A possible reason for these characteristics could be, that the S-100 protein is localized in astroglial cells, which in comparison to neurons are more resistent to ischaemia. There are no comparable data in the reviewed literature.

Skogseid found in his study, comparing patients with severe and moderate head injuries with patients with minor head injury, a correlation of NSE with the severity of injury assessed clinically and with the volume of contusion as estimated from CT [15]. We could not find a correlation of initial NSE and S-100 serum levels with GCS on admission and with CT classification of The Traumatic Coma Data Bank within our group of severely injured patients. Like Marshall *et al.* [10], we found a significant correlation of our CT findings with outcome. The GCS findings on admission showed in our population, probably due to the absolute number only, a strong trend with outcome.

We think that our results are encouraging for further studies on larger series to evaluate our results concerning the prognostic significance of NSE and S-100 after severe head injury and the possibility of these markers indicating secondary brain damage in the intensive care unit.

Conclusions

Patients with unfavourable outcome showed a significant higher serum concentration of S-100 within 5 hours after injury. However, our results show that the first serum concentration of NSE and S-100 are necessary to predict outcome after severe head injury. In our opinion the time course of marker concentrations, especially NSE, seems to give further information about secondary brain damage.

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Comments

Outcome is reported on discharge at a mean of 19 days after trauma. Although this may remain the primary endpoint for the evaluation as conducted by the authors I still maintain that outcome should be reported additionally at a fixed time point after injury, preferably three months or six months post-injury.

A further point of concern is the dichotomy of the Glasgow Outcome Scale into good and bad categories, listing patients with severe disability, moderate disability and good recovery, all as good outcome. There is general concensus currently concerning dichotomizing Glasgow Outcome Scale into favourable and unfavourable outcome, listing patients with favourable as those with moderate disability and good recovery and those with unfavourable as dead, vegetative and severely disabled. For purposes of a standardization I am very reluctant to accept a different dichotomy of the GOS as labelling this as good and bad. Maintaining the dichotomy into dead, vegetative on one side and severe disability, moderate disability and good recovery on the other side, is perhaps acceptable, but should then not be labelled good and bad outcome.

A further, maybe not so important point, and perhaps surpassing the context of this manuscript, concerns the CT classification of head injury according to Marshall. The problem is the classification of patients in the category of evacuated or non-evacuated mass lesions. In my earlier comments I stated that it was remarkable that no patients were listed in the category evacuated mass lesion. The authors respond with the remark that it concerns classification on the initial admission CT and patients were not operated on at that time. I may contend that speaking formally and very strictly their interpretation may be correct, but this was certainly not the intention of the original classification as proposed by Larry Marshall. Patients should be included in the category evacuated mass lesion when the CT scan shows an intracranial lesion of such proportions that operation is considered appropriate and scheduled. In this regard I may refer to the legend on Fig. 4 of the manuscript by Marshall *et al.* in the Journal of Neurosurgery, Volume 75, 1991 on page S17.

A. Maas

The authors have investigated neuron specific enolase (NSE) and S-100 protein following severe head injury in correlation with outcome. They studied 30 patients with a Glasgow Coma Score of less than 9 prospectively. Blood samples were taken on admission. Outcome was measured on discharge. Since patients with poor outcome had higher concentrations of S-100 than those with good outcome, the authors conclude that the first serum concentration of S-100 is predictive for outcome after severe head injury.

This is certainly an interesting report on two potential biochemical markers of the severity of brain trauma. Both have been proposed as such, and there are reports already that NSE serum levels correlate with the severity of injury or the contusion volume from CT scans. This has been cited by the authors and they are thus well aware of this general concept. Thus, one would expect that the study goes further. This, however, is not the case at the moment.

So far, only 30 patients have been studied. Regarding the wide spectrum of lesions and severity in head injury, this number is certainly low, and this is a preliminary report. At the moment, it is not possible to draw any general conclusions, and it is not possible to make valid and highly significant correlations in relation to outcome. The distinction between good versus poor outcome in comparison to average NSE and S-100 serum levels is rather poor, and only marginally significant as regards S-100. A major point of criticism is that outcome was determined on discharge. Meanwhile, it is widely accepted that outcome following severe head injury should be determined at least 6 months post injury, in order to obtain valuable information.

A. Unterberg

Answer from the authors:

We would not expect to find a single serum marker which is able to predict outcome at a follow-up period of six months, because the number of different factors influencing outcome after severe head injury are many. We would like to see S-100 as a marker predicting survival after severe head injury and to assess the severity of the injury. We think that our results are preliminary and a follow-up is under way.

All patients are admitted with sedation and relaxation, so the GCS is done at the scene of the accident. This classification is not always reliable; this is another reason to look for a further reliable prognosticator.

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