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Nerve growth factor expression correlates with severity and outcome of traumatic brain injury in children

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Abstract

Background—Secondary brain damage after traumatic brain injury (TBI) involves neuro-inflammatory mechanisms, mainly dependent on the intracerebral production of cytokines. In particular, interleukin 1 β (IL-1 β) is associated with neuronal damage, while interleukin 6 (IL-6) exerts a neuroprotective role due to its ability to modulate neurotrophins biosynthesis. However, the relationship between these cytokines and neurotrophins with the severity and outcome of TBI remains still controversial.

Aims—To determine whether the concentration of IL-1 β and IL-6 and neurotrophins (nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), glial-derived neurotrophic factor (GDNF)) in the cerebrospinal fluid (CSF) of children with TBI correlates with the severity of the injury and its neurologic outcome.

Methods—Prospective observational clinical study in a university hospital. CSF samples were collected from 27 children at 2h (Time T1) and 48h (Time T2) after severe TBI, and from 21 matched controls. Severity of TBI was evaluated by GCS and neurologic outcome by GOS. CSF concentrations of cytokines and neurotrophins were measured by immunoenzymatic assays.

Results—Early NGF and IL-1 β concentrations (T1) correlated significantly with the severity of head injury, whereas no correlation was found for IL-6, BDNF, and GDNF. Furthermore, higher NGF and IL-6 and lower IL-1 β expression at T2 were associated with better neurologic outcomes. No significant association was found between BDNF or GDNF expression and neurologic outcome.

Conclusions—NGF concentration in CSF is a useful marker of brain damage following severe TBI and its up-regulation, in the first 48 h after head injury together with lower IL-1 β expression, correlates with a favorable neurologic outcome. Clinical and prognostic information may also be obtained from IL-6 expression.

Keywords

Children; Cytokines; Neuroprotection; Neurotrophic factors; Traumatic brain injury

1. Introduction

Traumatic brain injury (TBI) is the most common cause of death and acquired disability among children and young adults in developed countries.¹ The clinical outcome of these patients is determined not only by the primary brain lesions, but also by the extent of secondary brain damage caused by excessive glutamate release, pathological increase of the intracellular calcium concentration, production of free oxygen radicals, lipid peroxidation, and above all the severity of the post-traumatic inflammatory response.² In fact, both experimental models and clinical studies have shown a strong association between secondary brain damage and neuro-inflammatory mechanisms, mainly dependent on the activation of T-cells and monocytes and the consequent intracerebral production of cytokines.^{3,4}

Interleukins 1 β (IL-1 β), IL-4 and IL-12 are critical mediators of post-traumatic neuro-inflammation and are associated with neurotoxicity and cortical neuronal damage.^{5–8} In contrast, increased expression of IL-6, IL-8, and IL-10 exert a neuroprotective activity,^{3,9,10} probably due to their ability to modulate the biosynthesis of nerve growth factor (NGF) and other neurotrophins like the brain-derived neurotrophic factor (BDNF) and glial-derived neurotrophic factor (GDNF), which play a key role in the recovery of injured brain neurons.^{11–15}

In animal models of experimental brain injury, exogenous administration of NGF and other neurotrophic factors has been shown to prevent or significantly reduce severe neurological deficits, apoptosis, and brain-cell death.^{16–19} The mechanisms underlying this neuroprotective role of NGF are not fully characterized, but several studies have shown its positive influence on cerebral blood flow, cellular Ca²⁺ homeostasis, and antioxidant activity.^{14–17} Inflammatory stimuli like hypoxia and TBI induce local NGF up-regulation, and cytokine-dependent NGF induction has been observed in the cerebrospinal fluid (CSF) following intra-ventricular infusion of IL-6 in animals with experimental brain injury.^{3,12}

Despite the mounting interest, the interactions between cytokines and neurotrophins in the central nervous system of children with TBI and their relationships with the severity of brain injury and clinical outcome are still unclear and controversial.^{20–22} Therefore, objective of the present study was to investigate the expression of the pro-inflammatory cytokine IL-1 β , the anti-inflammatory cytokine IL-6, and the neurotrophic factors NGF, BDNF and GDNF in the CSF of children with TBI, in order to clarify whether these factors can be useful for the assessment of clinical severity and/or the prognosis of neurologic outcome.

2. Methods

2.1. Study population

We conducted a prospective observational clinical study in children admitted for TBI to the Pediatric Intensive Care Unit (PICU) of the “Agostino Gemelli” Hospital in Rome, Italy. We included in this study only children with isolated head injury admitted within 2h from the trauma. Glasgow Coma Scale (GCS) scores of 8, 9–12 and > 12 were used to define severe, moderate and mild head injury, respectively. Only children with severe head injury (i.e., GCS 8) were enrolled in this study.

All patients were kept in supine position with 30° head elevation. Central venous and arterial line catheters were used to monitor blood pressure. Basal metabolic parameters were monitored continuously to prevent hyperglycaemia or other metabolic derangements. Fever was treated with antipyretics and cooling blankets, and core (oesophageal) temperature was maintained between 36 and 37 °C. The standard intensive care management for all children

included the administration of intravenous midazolam (2–5 µg/kg/min) and remifentanyl (0.25 µg/kg/min), and mild mechanical hyperventilation to maintain PaCO₂ between 35 and 38 mmHg and PaO₂ between 90 and 100 mmHg. For children with intracranial pressure (ICP) above 20 mmHg, hyperventilation and oxygenation were controlled using target values of PaCO₂ 32–34 mmHg and PaO₂ 100 mmHg, and a head CT scan was performed.

In children with refractory intracranial hypertension, after performing a second head CT scan, we monitored cerebral oxygen extraction (CEO₂) using an internal jugular vein bulb catheter (CEO₂ = SaO₂-SjO₂; normal SjO₂ >55%<75%; hyperemic state >75%, oligoemic state <55%). In all children ICP was continuously monitored and CSF was drained using an intraventricular catheter. In case of increased ICP resistant to aggressive therapy with 18% mannitol and hyperventilation, we induced barbiturate coma, monitoring CEO₂ as well as cerebral blood flow velocity by transcranial Doppler sonography. Therapy was geared towards the maintenance of a cerebral perfusion pressure (CPP = mean arterial pressure-intracranial pressure) of 70 mmHg (50 mmHg in infants).

To measure cytokines and neurotrophins expression at 2 h (T1) and 48 h (T2) after brain injury, we collected CSF samples into a closed, sterile system using the same intraventricular catheter set for ICP monitoring. All CSF samples were centrifuged for 10 min at 5000 rpm, and the supernatant was immediately stored at -70 °C until analysis. As controls, we used CSF samples collected from children who underwent lumbar puncture to rule out meningitis suspected from clinical and laboratory findings (fever, mental state alteration, and acute phase reactant elevation). None of these patients had evidence of meningitis (as confirmed by negative cultures, chemical analysis and lack of pleocytosis). TBI patients and control subjects were matched for age, sex and weight, respectively. Outcomes were assessed 6 months after TBI using the Glasgow Outcome Score (GOS) (that represents a “global” and not specific outcome score), which assigns a score of 1 to children who died, 2 to persistent vegetative state, 3 to severe neurologic deficits, 4 to mild neurologic deficits, and 5 to completely healthy children. Using the GOS, we considered to dichotomise the clinical outcome in two categories: poor outcome when the GOS was between 1 and 3, and good outcome when the GOS was 4 or 5.

This study was approved by the University’s institutional review board and ethical committee, and the parents of all participating children provided written informed consent.

2.2. Cytokine assays

IL-1β and IL-6 were measured using a commercial two-site immunoenzymatic kit (Euroclone Life Science, Milan, Italy). Sensitivity was 1pg/mL for IL-1β and 0.70 pg/mL for IL-6. No cross-reactivity or interference with related interleukins was observed. All assays were performed in triplicate and results were expressed as pg/mL.

2.3. Neurotrophin assays

NGF levels were measured with a highly sensitive two-site immunoenzymatic assay following manufacturer’s instructions (R&D Systems, Minneapolis, MN, USA). Sensitivity was 1 pg/mL and cross-reactivity with other neurotrophic factors was less than 0.3%. All assays were performed in triplicate and results were expressed as pg/mL.

BDNF was quantified using a two-site immunoenzymatic assay from Promega (Madison, WI, USA). As with the NGF assay, Ninety-six-well plates were coated with 100µL/well of monoclonal anti-mouse BDNF antibody and incubated overnight at 4°C. The plates were then washed three times with buffer, and samples were incubated in the coated wells (100 µL/well) for 2 h at room temperature. After additional washes, the antigen was incubated with anti-human BDNF antibody for 2 h at room temperature. The plates were washed again

with buffer and then incubated with an anti-chicken IgY HRP for 1h at room temperature. After another washing cycle, the plates were incubated with TMB/peroxidase substrate for 15min, and 1M phosphoric acid was added to the wells (100µL/well). The colorimetric reaction product was measured at 450nm using an ELISA reader (Dynatech MR 5000, Alexandria, VA, USA). BDNF concentrations were interpolated from a BDNF standard curve ranging from 7.8 to 500 pg/mL of purified mouse BDNF calculated under similar conditions in each assay. Sensitivity of this assay was 3pg/mL and cross-reactivity with other neurotrophins was less than 3%. All assays were performed in triplicate and BDNF concentration was expressed as pg/mL.

GDNF was quantified using a two-site immunoassay kit from Promega similar to that used for BDNF. 96-well plates were coated with 100 µL/well of monoclonal anti-GDNF antibody. After overnight incubation at 4°C, the antibody was removed from the plates and the samples were incubated in coated wells (100µL/well) for 6h at room temperature. The plates were then washed five times with buffer, and the antigen was incubated overnight with polyclonal anti-human GDNF antibody at 4°C. The plates were washed again with buffer and incubated with anti-chicken IgY HRP conjugate for 2h at room temperature. The remaining steps of the procedure were identical to those used for BDNF. The plates were incubated with a TMB/peroxidase substrate solution for 15min, and 1M phosphoric acid was added (100 µL/well). The colorimetric reaction product was measured at 450nm. GDNF concentrations were interpolated from a GDNF standard curve ranging from 15.6 to 1000 pg/mL of purified human GDNF. The sensitivity of this assay was 3pg/mL and cross-reactivity with other related neurotrophins was less than 5%. All assays were performed in triplicate and GDNF concentration was expressed as pg/mL.

2.4. Statistical analysis

Mean concentrations of cytokines and neurotrophins measured in the CSF of children with TBI were compared with controls using the Student's t-test. To provide a synthetic description of the association of cytokines and neurotrophins concentrations with the GCS scores assessed upon admission, we used bi-dimensional scatter plots where the estimated linear regression line was superimposed. The estimated linear regression was calculated considering GCS as a continuous variable, although it is not. To evaluate the association of cytokines and neurotrophins concentrations with GOS assessed at 6 months after TBI, we constructed box plots stratified by class of GOS (GOS = 3 = poor outcome; GOS > 3 = good outcome). To evaluate if there was a statistically significant difference between the two outcome groups for any of the considered parameters, we used the Student's t-test. Multiple ordinal logistic regression analysis was used to test the association of GOS with the change of each parameter having simultaneously adjusted for the variation of the others. In this analysis, we excluded GDNF because we found an extremely high correlation (0.98) with the BDNF variation. In this analysis, we also did not include the GCS score on admission because this value was almost always highly predictive of GOS at discharge. A *p*-value < 0.05 was considered significant. Statistical analysis of the data was performed using Stata 8.0 software (Stata Corporation, College Station, TX, USA).

3. Results

3.1. Demographic and clinical data

Over the 18 months of this study, 27 children with severe TBI (14 males and 13 females) ranging from 1.3 years to 15.6 years of age (mean 8.6±4.5 years) were admitted to the PICU within 2h from the trauma. All children had suffered an isolated head injury caused by motor vehicle accident (*n* = 23), fall (*n* = 3), or domestic accident (*n* = 1). The control group consisted of 21 children who underwent lumbar puncture to rule out meningitis age- and

weight-matched with the TBI patients. Table 1 shows the demographic data recorded upon hospital admission, together with GCS scores on admission, GOS at 6 months post-TBI, and the concentrations of cytokines and neurotrophins measured in the CSF at 2h (T1) and 48h (T2) after the trauma. GOS was 1 in seven patients, 2 in one patient, 3 in three patients, 4 in five patients, and 5 in 11 patients.

3.2. Cytokines and neurotrophins expression

CSF concentrations of NGF and IL-6 measured 2h post-trauma (Fig. 1) were dramatically increased in patients with TBI compared to controls, by 23- and 10-fold, respectively ($p < 0.01$). Statistically significant, albeit much smaller, differences were measured also for BDNF and IL-1 β ($p < 0.01$), whereas there was no statistically significant difference in GDNF concentration between patients vs. controls ($p = 0.23$).

A similar profile of neurotrophins and cytokines expression was observed in TBI patients 48h post-trauma (Fig. 2). Further increase was noted in the concentrations of NGF and IL-1 β , whereas BDNF, GDNF, and IL-6 concentrations decreased from T1 to T2.

3.3. Correlation with severity

The scatter plots in Fig. 3 show the linear regression analysis of cytokines and neurotrophins concentrations measured at T1 and GCS scores recorded upon admission to the PICU. NGF ($p < 0.01$) and IL-1 β ($p = 0.01$) concentrations correlated significantly with GCS scores. In contrast, no significant correlation was found between IL-6 ($p = 0.17$), BDNF ($p = 0.28$), or GDNF ($p = 0.29$) concentrations and GCS scores.

3.4. Correlation with outcome

The box plots in Figs. 4–6 illustrate the concentrations of cytokines and neurotrophins in the CSF measured at T1 and T2, as well as their differential ($\Delta T2-T1$), sorted by GOS category. NGF concentrations measured at T1 were significantly lower in patients with better neurologic outcomes at 6 months post-trauma ($p < 0.01$; Fig. 4). However, these patients also had significantly higher NGF ($p = 0.02$) and IL-6 ($p < 0.01$) together with lower IL-1 β ($p < 0.01$) concentrations measured at T2 (Fig. 5). When evaluating the differentials between T1 and T2 (Fig. 6), we found that patients with better outcomes had smaller increments in IL-1 β expression ($p < 0.01$), but also significantly stronger NGF and IL-6 up-regulation ($p < 0.01$). In a multiple ordinal logistic regression where the outcome was the GOS and the potential predictive variables were the changes in cytokines and neurotrophins concentrations between T1 and T2 (Table 2), we found that only the change in NGF, having simultaneously adjusted for the other parameters, was significantly associated with favorable neurologic outcome. It is of note that in further model where GCS at admission was added together with the changes in cytokines expression, it was not possible to disentangle the effect of GCS at admission from that of the other parameters due to high correlation between them (see Fig. 3).

4. Discussion

This study shows that children with severe head trauma develop within 2 h a remarkable increase in the CSF concentrations of NGF and IL-6, with smaller but significant increase also in BDNF and IL-1 β concentrations. NGF and IL-1 β increase further at 48h post-trauma, whereas the other markers tend to decline. The expression of NGF, and to a lesser degree IL-1 β , early after severe head trauma correlates with the severity of neurologic compromise assessed upon admission. Also, concurrent strong up-regulation of NGF and IL-6 relative to low IL-1 β expression in the CSF early after head trauma seems to better predict neurologic outcome.

Inflammation has been postulated to contribute to the extent of secondary brain damage in patients with head trauma through a number of mechanisms including complement activation, neutrophilic infiltration, local cytokines production, and increased expression of neurotrophic factors.^{4,23,24} However, the specific relationship of cytokines and neurotrophins with the severity of brain injury and with the neurologic outcome remains still controversial. Recent studies have shown that increased levels of IL-1 β are associated with increased severity and poor outcome in patients affected by meningitis, hypoxic-ischemic encephalopathy, and traumatic head injuries.^{25–27} The results of our study are consistent with those findings, confirming that an early increase of IL-1 β concentration in the CSF is significantly correlated with both the severity of head injury and poor neurologic outcome. The significant association between increased CSF levels of IL-1 β at 2h post-trauma and low GCS suggests that this cytokine may be a molecular marker of head injury severity. Moreover, the association between progressive increase of IL-1 β after injury and poor outcome suggests that the local production of this cytokine contributes to the extent of secondary brain damage in patients with head trauma.

Several mechanisms can explain the relationship between IL-1 β up-regulation and poor neurologic outcome in children with severe TBI. This cytokine exhibits, synergistically with TNF α , a direct neurotoxic effect increasing production and release of nitric oxide synthase, free oxygen radicals, and excitatory amino acids.^{28,29} Higher concentrations of IL-1 β have been shown in the CSF of newborns with perinatal asphyxia, and this up-regulation has been linked to the degree of encephalopathy and to the severity of subsequent neurological outcomes, probably because this cytokine can negatively regulate neurogenesis and mechanisms of neuronal repair following injury.^{27,30,31} Our study confirms that IL-1 β up-regulation after a traumatic insult represents an important biochemical marker of the severity of brain damage, and that its increased expression is significantly associated with poor neurological outcomes, confirming the role of IL-1 β in inducing neuronal injury and extensive brain damage.^{31,32}

Higher NGF levels in the CSF measured 2h after the trauma are significantly associated with the severity of TBI. Furthermore, we have shown a significant correlation between early post-traumatic up-regulation of both NGF and IL-6 and good neurological outcomes, confirming the neuroprotective role exerted by both these factors.^{9,33,34} Our findings are also consistent with the timing of neurotrophic factor expression in the CSF of patients with head trauma and in animal models of experimental brain injury,^{21,22} suggesting that this early event is upstream from the complex cascade of local inflammatory host response after TBI. NGF biosynthesis in the injured brain can be induced by IL-6 through its effects on neurons, microglia and astrocytes, as similar neuroprotective mechanisms have been recognized both in traumatic and ischemic lesions.^{35–37} Furthermore, IL-6 up-regulation has been found using cerebral micro dialysis probes in head-injured adult patients³⁴ and our results are consistent with this study, confirming that IL-6 is an endogenous neuroprotective cytokine produced in response to brain injury. Also neurotrophic factors have been shown to play a neuroprotective role in different CNS dysfunctions.^{38,39}

In our study, the expression of NGF, BDNF and GDNF was differentially regulated in the CSF of children with severe TBI, suggesting that these factors act on different target cells and serve different functions in post-traumatic injury. In particular, NGF up-regulation in our patients was remarkably stronger than the changes in BDNF and GDNF, and it was significantly correlated with clinical severity and outcomes. Although BDNF and GDNF exhibited a small increase, their expression in the CSF did not correlate with the severity of brain injury or with the neurologic outcome, as previously reported.²⁰ NGF up-regulation was strongly associated with the recovery of cerebral functions after injury, whereas patients who suffered brain death or persistent vegetative state were characterized by flattening of

the NGF concentration curve in the CNS and decreased levels from T1 to T2, suggesting that a severely damaged brain is unable to adequately produce NGF in response to the injury. In contrast, children with a higher GOS had increased NGF concentration at T2, which was predictive of a good clinical outcome. Indeed, our statistical analysis shows that, among all molecular determinants studied, NGF is the most associated factor of neurologic outcome. Although GCS still remains the most important clinical score to predict clinical outcome, this analysis provides new information about the molecular mechanism of brain damage. Because of the low sample size and of the high correlation with GCS, it remains the difficulty on understanding whether these molecular mechanisms can provide an independent contribution on predicting the outcome.

Only limited information is available concerning NGF expression in the CSF of patients with severe TBI.^{20,21,40} In these studies, the levels of NGF increased rapidly and appeared to be enhanced in hypoxic-ischemic brain tissue and in the early phases after a traumatic insult. This process could represent a defensive mechanism of the brain against the effects of cerebral hypoxia and excitotoxicity, the two primary mechanisms of neuronal death following brain injury.^{41,42} NGF up-regulation in traumatized patients plays a key role in the inflammatory response and can have a beneficial impact on the regenerative capacity of the injured brain. There are several hypotheses to explain why NGF might exert neuroprotective effects in patients with severe brain injury, in whom an important impairment of cholinergic brain functions and extensive neuronal loss have been reported.^{43,44} NGF may arrest the ongoing degeneration of cholinergic projections to the cerebral cortex, as previously reported in patients with Alzheimer's disease.⁴⁵ In addition, NGF leads to up-regulation of central nicotinic receptors, improving cortical blood flow and counteracting post-traumatic cerebral ischemia and neuronal death.^{46,47} Recently, it has been reported that intraventricular NGF administration improves neuronal survival in infants suffering from hypoxic-ischemic brain injury, and that neurotrophic factors promote striatal neurogenesis and cellular proliferation in traumatized brain tissues.^{48,49}

In conclusion, our study shows differential expression of IL-1 β , IL-6, and neurotrophic factors in the CSF of children early after severe TBI. The observed pattern of cytokine and neurotrophin expression after TBI may reflect an endogenous attempt at neuroprotection against the biochemical cascades triggered by the traumatic insult. The up-regulation of IL-1 β and NGF represents a particularly important biochemical marker of severe brain damage as it is significantly associated with the severity of head injury. Whereas the IL-1 β up-regulation correlates significantly with poor neurological outcome for its pro-inflammatory and direct neurotoxic effects, the up-regulation of IL-6 and NGF is predictive of favorable outcome for their role in neuronal repair and survival. Although additional studies are needed for a better understanding of IL-6/NGF modulation, intraventricular NGF administration may become a promising strategy for the treatment of children with severe TBI.

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Fig. 1. Neurotrophins and cytokines concentrations measured in the CSF of children with severe brain injury 2h after trauma compared to controls.

Fig. 2.
Comparison of neurotrophins and cytokines concentrations in the CSF of children with severe brain injury measured at 2h (T1) and 48h (T2) post-trauma.

Fig. 3.

Scatter plots and estimated linear regression of cytokines and neurotrophins concentrations measured in the CSF at T1 with Glasgow Coma Scale on admission. Only NGF and IL-1 β concentrations correlated significantly with GCS scores.

Fig. 4. Box plots of cytokines and neurotrophins concentrations measured in the CSF at T1 sorted by category of Glasgow Outcome Score. Only NGF expression was statistically associated with neurologic outcome.

Fig. 5. Box plots of cytokines and neurotrophins concentrations measured in the CSF at T2 sorted by category of Glasgow Outcome Score. IL-1 β over-expression was significantly associated with poor neurologic outcome, while IL-6 and NGF over-expression was significantly associated with a favorable outcome.

Fig. 6.

Box plots of cytokines and neurotrophins changes between T1 and T2 sorted by category of Glasgow Outcome Score. IL-1 β up-regulation was significantly associated with poor neurologic outcome (GOS ≤ 3), whereas up-regulation of IL-6 and NGF was significantly associated with favorable outcome (GOS > 3).

Table 1

Summary of demographic, clinical and laboratory data

Patient	Age (years)/sex	GCS	GOS	NGF		BDNF		GDNF		IL-1 β		IL-6	
				T1	T2	T1	T2	T1	T2	T1	T2	T1	T2
1	13.6/F	8	5	248	446	61	24	51	14	5	6	249	387
2	4/F	8	5	280	792	47	16	37	6	3	3	360	460
3	8.2/F	7	5	260	795	48	30	38	20	3	4	378	486
4	3.4/F	8	5	297	726	51	39	41	29	4	5	365	435
5	5.4/F	8	5	295	730	18	12	8	2	4	6	372	490
6	8/M	7	5	235	760	12	39	2	29	5	6	378	486
7	1.3/M	7	5	296	730	46	31	36	21	5	7	280	383
8	2/M	8	5	198	229	3	7	4	8	5	10	355	283
9	15/M	8	5	246	327	3	3	5	7	3	3	351	358
10	13.2/F	7	5	171	252	51	45	41	35	7	8	192	259
11	15/M	7	5	298	516	11	17	10	16	6	6	345	450
12	15.6/M	6	4	250	392	110	73	90	53	6	9	323	377
13	6.2/F	7	4	278	480	86	22	76	12	7	10	325	372
14	6/M	7	4	290	693	27	20	17	10	14	18	378	373
15	7/F	7	4	297	492	3	9	5	11	4	8	390	390
16	5.8/F	5	4	287	260	3	3	5	5	14	16	301	341
17	12.7/M	5	3	370	398	41	54	31	44	7	9	320	290
18	15.2/F	5	3	380	390	11	9	10	8	7	21	288	165
19	11/F	6	3	381	328	4	11	6	13	3	6	319	109
20	3/F	4	2	270	290	6	21	6	20	8	9	372	185
21	6/M	4	1	703	486	4	3	6	5	3	4	354	114
22	3/M	4	1	780	330	22	13	12	3	7	13	394	112
23	14/M	3	1	705	260	11	11	13	13	7	14	392	102
24	9/M	4	1	703	400	16	14	6	4	8	24	412	116
25	9/M	3	1	810	260	18	12	8	7	7	21	398	115
26	12.6/F	4	1	710	482	17	15	18	12	8	20	394	110
27	8.2/M	4	1	553	436	68	70	38	40	10	21	303	178

Note: Glasgow Coma Scale scores were assessed on admission; Glasgow Outcome Score at 6 months after traumatic brain injury. Neurotrophins and cytokines concentrations measured in the CSF at 2 h (T1) and 48 h (T2) after traumatic brain injury are expressed in pg/mL.

Table 2

Estimated adjusted coefficients for changes in CSF concentrations of cytokines and neurotrophins at time T1 (a), at time T2 (b), and from time T1 to time T2 ($\Delta T2-T1$) (c) from an ordinal logistic regression having as outcome Glasgow Outcome Scores at 6 months after TBI

	Coefficient	95% CI		pvalue
(a) T1 values (pg/mL) (per 1 unit increase)				
CSF NGF	−0.03	−0.06	−0.01	0.01
CSF BDNF	0.02	−0.10	0.15	0.71
CSF GDNF	−0.06	−0.21	0.10	0.49
CSF IL1β	−0.50	−0.89	−0.11	0.01
CSF IL6	−0.01	−0.04	0.02	0.56
(β) T2 values (pg/mL) (per 1 unit increase)				
CSF NGF	0.00	−0.01	0.01	0.77
CSF BDNF	−0.02	−0.22	0.18	0.82
CSF GDNF	0.00	−0.28	0.28	0.99
CSF IL1β	−0.20	−0.39	−0.01	0.04
CSF IL6	0.02	0.01	0.03	0.00
(c) ΔT2—T1 (pg/mL)				
CSF NGF ΔT2—T1 (per 10 unit increase)	0.08	0.00	0.15	0.04
CSF BDNF ΔT2—T1 (per 10 unit increase)	0.11	−0.37	0.60	0.65
CSF IL1β ΔT2—T1 (per 1 unit increase)	−0.24	−0.51	0.03	0.08
CSF IL6 ΔT2—T1 (per 10 unit increase)	0.09	−0.05	0.23	0.20

Early (time T1) NGF and IL-1 β expression and late (time T2) IL-1 β and IL-6 expression were independently associated with outcome. Considering these factors, only the increase of NGF from time T1 to time T2 was significantly associated with good outcome in children with severe TBI. *Note:* In part (c), CSF GDNF $\Delta T2-T1$ was not included in this analysis because of the extremely high correlation (0.98) with CSF BDNF $\Delta T2-T1$.