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Time course of cerebrospinal fluid inflammatory biomarkers and relationship to 6-month neurologic outcome in adult severe traumatic brain injury

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ABSTRACT

Objective: Activation of the inflammatory cascade is a known pathophysiologic process in severe traumatic brain injury (TBI) with yet non-standardized scientific data regarding relationship to outcome. The understanding of the time course of expression of cerebrospinal fluid (CSF) biomarker levels following severe TBI is an important step toward using these biomarkers to measure injury severity and/or early response to therapeutic interventions. The objective of the current study is to report the time course and values of a battery of CSF inflammatory biomarkers following severe TBI in our reasonably sized patient cohort.

Patients and methods: Our patient cohort consists of 32 consented patients, who met the study's inclusion criteria for data collection from 2000 to 2010. The time course and values of a battery of CSF biomarkers (IL-1β, IL-6, TNF-α, IFN-γ, IL-12p70, IL-10, and IL-8) following severe TBI in this patient cohort was characterized. Additionally, the correlation of biomarker concentration with 6-month neurological outcome was assessed. Serial CSF sampling through an external ventricular drain was performed over the first five days following injury. Concentration of a panel of inflammatory biomarkers (IL-1β, IL-6, TNF-α, IFN-γ, IL-12p70, IL-10, and IL-8) were evaluated using Meso Scale Discovery's Multi-Array technology. Glasgow Outcome Scale (GOS) score at six months following injury was dichotomized into poor outcome (GOS 1-3) and favorable outcome (GOS 4-5). Statistical analyses were performed using Kruskal-Wallis test and linear regression analysis.

Results: The result shows that CSF concentrations of inflammatory biomarkers had a significant association with 6-month neurological outcome (p-values ≤ 0.05 for each marker), with the favorable outcome group having lower concentrations of these biomarkers on average, in comparison to the poor neurologic outcome group over the first five days after TBI. All inflammatory biomarkers decreased to normal levels by post-trauma day 5, except for IL-6 and IL-8. Upregulation and increased expression of key inflammatory markers following severe TBI were significant predictors of worse 6-month neurologic outcome. Additionally, post-trauma day 5 concentrations of IL-6 and IL-8 remained elevated over normal CSF values.

Conclusion: The study shows that inflammatory biomarkers in CSF are potential biomarkers of injury severity and progression and/or recovery; they could prove beneficial in the future assessment of injury severity and response to therapy after severe TBI.

1. Introduction

Traumatic brain injury remains one of the leading culprits of death around the world. To date, effective neuroprotective agents and prognostic evaluation of severe traumatic brain injury (TBI) remains elusive [1–3]. A major challenge of TBI clinical trial design has been the reliance on 6-month neuropsychological testing as the primary outcome measure [4]. Numerous scientific articles

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have published data showing that assessment of both central and peripheral biomarkers may prove essential in establishing injury severity, monitoring response to treatment, and estimating prognosis in patients with severe TBI. TBI has been shown to alter the measured concentrations in cerebrospinal fluid (CSF) of multiple CNS proteins [4–6]. The importance of inflammation on the progression of TBI-associated secondary injury has been the focus of numerous studies [1]. IL-1β, IL-6, TNF-α, IFN-γ, IL-12p70, IL-10, and IL-8 are a group of cytokines that have been well described in the literature as far as their involvement in the secondary injury that occurs in the heterogenous injury that is TBI. The aforementioned cytokines, we believe could offer further insight on ways to alter the early progression of the inflammatory response in TBI [7].

The current study reports the time course and values of a battery of CSF inflammatory biomarkers following severe TBI in a reasonably sized patient cohort. We further correlated biomarker concentration with 6-month neurological outcome.

2. Methods

2.1. Study population

Subjects included in this study were extracted from a large prospectively collected database and biospecimen repository within the Brain Trauma Research Center (BTRC), approved by the University of Pittsburgh Institutional Review Board, which included patient and/or family consent for data collection for greater than 1000 patients. The consenting process was conducted by physicians and nurses who are part of the neurotrauma research staff at the University of Pittsburgh Medical Center. The study cohort consisted of 32 patients who were consented and met the study's inclusion criteria for data collection from 2000 to 2010. This is a convenience sample of patients from a larger consecutively collected database, which is the BTRC. Patient age, sex, and neurologic injury severity (dictated by Glasgow Coma Scale {GCS} score at admission or when clinical deterioration occurred) were collected. Additional demograhic data regarding mechanism of injury, type of injury, injury severity score (ISS), intensive care unit (ICU) length of stay, hypothermia treatment (from patients involved in a clinical trial), death and 6-month survival status also were obtained. All patients in the study had severe TBI classification (Glasgow Coma Scale [GCS] score of ≤8 on admission or when clinical deterioration occurred). The patients were eligible regardless of their mechanism of injury, but were excluded from the study if they did not have an EVD placement,age less than 17 or greater than 80, their GCS was >8, or if they died prior to post-trauma day 5 because the authors wanted to have at least a 5 day collection period to evaluate the time course of both intracranial pressure and CSF physiology in the acute phase of trauma.

2.2. CSF and data collection

Physiologic data (vital signs, intracranial pressure, etc) was collected every minute and serial CSF samples collected every 6 h from all consented subjects admitted to UPMC Presbyterian Hospital with a severe traumatic brain injury (initial Glasgow Coma Scale [GCS] score ≤ 8). As part of the protocol, serial CSF samples are collected for the duration of EVD placement up to seven days. All subjects underwent an assessment of Glasgow Outcome Scale (GOS) score at 6 months following injury. GOS is a global 5 category scale which objectively classified outcomes of TBI patients at different time periods in the following manner: 1 (dead), 2 (vegetative state), 3 (severe disability), 4 (moderate disability), and 5 (good disability). This data was collected 3, 6, 12, and 24 months post-trauma by certified neuropsychologists within the brain trauma research staff.

All samples were frozen upon collection in the intensive care unit (which was usually dependent on time patient presentation and placement of external ventricular drain, usually within 1–12 h of presentation with average being 2 h) then thawed once in order to aliquot into 0.5 ml samples for storage in the BTRC neurotrauma laboratory at −80 °C. All CSF samples used in inflammatory biomarker appraisal were supplemented with 1% bovine serum albumin (BSA) and centrifuged at 13,000*g* at 4 °C for two minutes. Inflammatory cytokines/chemokines (IL-1β, IL-6, TNF-α, IFN-γ, IL-12p70, IL-10, and IL-8) were measured at the picogram concentration level using Human Pro-inflammatory 7-Plex Ultrasensitive kit (Meso Scale Development, Gaithersburg, MD USA) following manufacturer's instructions. Subsequently, the plates were read using a SECTOR Imager 2400. The total 24 h CSF sample, collected in 6 h increments from consented patients after placement of external ventricular drain, were used for analysis of daily biomarker concentrations. The daily values of each patient's CSF

Table 1
Patient demographics and injury characteristics.

Sample Size (n)	32
Age *(mean ± SD)*	31.0 ± 16.0
Sex – male *(n (%))*	22.0 (68.8)
GCS *(mean ± SD)*	6.0 ± 1.6
GOS *(mean ± SD)*	3.0 ± 1.4
GCS Classification	
GCS 3	4
GCS 4	6
GCS 5	6
GCS 6	4
GCS 7	10
GCS 8	2
Mechanism of Injury (*n*)	
Falls	5
MVA	21
MCA/ATV	3
MV/Ped	3
GOS Classification	
GOS 1	7
GOS 2	2
GOS 3	9
GOS 4	9
GOS 5	5
Types of Injury (n)	
SAH	6
SDH	4
EDH	1
IPH	1
SAH/SDH	2
DAI	1
Multi-comp	13
CTX	2
No bleed	1
Cerebral Edema	1
ISS (mean ± SD)	34.3 ± 10.3
Length of ICU stay (mean ± SD)	20.4 ± 12.4
Ventriculitis (n (%))	2 (6.25)
Hypothermia Treatment (*n* (%))	4(12.5)[a]
Survival at 6 months (*n* (%))	25 (78.1)
Death at 6 months (*n* (%))	7 (22.9)
Respiratory Failure	3
Brainstem Herniation	1
Neurological Deterioration	2
Sepsis	1

MVA: Motor vehicle Accident; MCA: Motorcycle Accident; ATV: All-terrain vehicle; MV/Ped: Pedestrian struck by motor vehicle; SAH: Subarachnoid Hemorrhage; SDH: Subdural Hematoma; EDH: Epidural hematoma; IPH: Intraparanchymal Hemorrhage; DAI: Diffuse Axonal Injury; Multi-comp: Multi-compartmental Hemorrhage; CTX: Cerebral Contusion.

[a] Note: These 4 patients treated with hypothermia where part of a clinical trial conducted at University of Pittsburgh Medical Center.