Journal of Neuroimmunology 377 (2023) 578020

Contents lists available at ScienceDirect

Journal of Neuroimmunology

journal homepage: www.elsevier.com/locate/jneuroim

Prolonged and intense neuroinflammation after severe traumatic brain injury assessed by cerebral microdialysis with 300 kDa membranes

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ARTICLE INFO

Keywords:
Brain metabolism
Brain injuries
Traumatic / pathology
Neuroinflammation
Microdialysis

ABSTRACT

Background: A neuroinflammatory response that may lead to edema and secondary brain damage is elicited in severe traumatic brain injury (TBI). Previous studies using microdialysis (MD) membranes with 100 k Dalton (kDa) cut-off found a transient intracerebral release of cytokines and chemokines without significant correlations to clinical course, intracranial pressure (ICP) or metabolites. In this study, a (300 kDa) MD probe was used to measure the levels of cytokines and chemokines in relation to ICP and metabolites.

Methods: Seven patients with severe TBI received 2 MD catheters. In four patients sufficient dialysate could be retrieved for analysis from both catheters. MD samples were analyzed bedside, then frozen and analyzed for chemokines and cytokines using a multiplex assay (Mesoscale Discovery).

Results: MD sampling was performed from 9 to 350 h. In total, 17 chemokines and cytokines were detected. Of these, IL-6, IL-8, IP-10, MCP-1 and MIP-1β were consistently elevated, and investigated further in relation to metabolites, and ICP. Levels of chemokines and cytokines were higher than previously reported from TBI patients, and partially higher than those reported in patients with cytokine release syndrome.

There were no significant differences between the two catheters regarding cytokine/chemokine concentrations, except for IL-6 which was higher in the peri-contusional area. No correlation with metabolites and ICP was observed. No significant increase or decline of chemokine or cytokine secretion was observed during the study period.

Conclusion: Our data suggest that cytokine and chemokine levels reflect a perpetual, potent and pan-cerebebral inflammatory response that persists beyond 15 days following TBI.

1. Introduction

Traumatic brain injury (TBI) is a global epidemic with high rates of morbidity and mortality, despite advances in prevention and therapy in developed countries(Maas et al., 2017). TBI results in macroscopic tissue alterations as hemorrhage, lacerations and contusions, often appearing simultaneously. Microscopically, cellular damage, intra- and extracellular edema and various forms of cell death are observed. On the molecular level, hypoxic, damaged or dying cells release a plethora of mediators that will orchestrate an evolving secondary brain damage (Jha et al., 2019; Ladak et al., 2019).

Sterile inflammation, defined as an inflammatory cascade initiated by the release of disease associated molecular patterns (DAMPs), has been associated to both the events that lead to secondary brain damage but also in the evolving tissue regeneration and degeneration after the trauma (Huber-Lang et al., 2018).

Sterile inflammation is the result of a cascade initiated by release of DAMPs, such as HMGB1, mDNA, IL-16 and IL-33 from stressed or dying cells, followed by outflow of pro-inflammatory and anti-inflammatory cytokines from resident or recruited cells. Additionally, other inflammatory mediators such as complement factors, kinins, reactive oxygen and nitrogen species play a role in inflammatory secondary brain damage (Bains and Hall, 2012; Tisdall et al., 2013). In the brain the inflammatory cascade is purported not only by invading inflammatory cells but also by brain resident cells with inflammatory capacity as microglia, neurons and astrocytes (Wofford et al., 2019)However, Inflammation can also promote tissue healing and regeneration after TBI (Maas et al., 2017; Zwir et al., 2020).

Sterile inflammation has been implicated in the evolution of cerebral edema that in turn results in raised intracranial pressure with ensuing

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https://doi.org/10.1016/j.jneuroim.2023.578020
Received 16 November 2022; Received in revised form 4 January 2023; Accepted 13 January 2023
Available online 18 January 2023

disturbances in perfusion and diffusion that lead to further tissue damage resulting in severe morbidity or death (Cederberg and Siesjo, 2010; Jha et al., 2019; Needham et al., 2019; Tucker et al., 2017).

Thus, there is an increasing interest in attenuating inflammation in TBI, but yet no successful interventions have been performed (Corps et al., 2015; Huber-Lang et al., 2018).

Clinical data supporting the role of inflammation in TBI have come from measurement of inflammatory mediators, mostly during intensive care treatment, imaging and postmortem studies. The release of DAMP mediators and inflammatory cytokines/chemokines after TBI can be measured in blood, cerebrospinal fluid (CSF), brain tissue and from the brain extracellular fluid, the latter by microdialysis (MD) (Thelin et al., 2017). In the present literature there is evidence that a gradient for most inflammatory mediators from brain to periphery exists, supporting the notion of local CNS production after TBI (Giorgi-Coll et al., 2017). Furthermore, it has been reported that the inflammatory response to TBI is different to that of multi-trauma and that this affects outcome(Rowland et al., 2020).

Cerebral MD, originally devised for the analysis of brain metabolites, makes sampling of brain extracellular fluid possible for subsequent analysis of inflammatory mediators. Microdialysis data from experimental and clinical TBI have displayed heterogenous results. However, some mediators such as IL-6, IL-8, MIP-1β, MCP-1 and IP-10 are repeatedly detected, in addition to numerous others found at lower concentrations that include IL1-b, GM-CSF, IL-2, IL-7 and IFNα (Zeiler et al., 2017)Due to the use of various designs of MD catheters and multiple platforms for analysis, the absolute values of the specific inflammatory mediators diverge between reports (Giorgi-Coll et al., 2017; Hillman et al., 2007). Due to intrinsic features of MD catheters, only a fraction of the actual cytokine levels is recovered and analyzed adding to variability. As the size of many inflammatory mediators is higher than the operating cut-off of standard 20 k Dalton (kDa) catheters, catheters with larger cut-off such as 100 kDa, have mostly been used. Although larger pore size catheters would theoretically give a better recovery, they also introduce the risk of ultrafiltration (Snyder et al., 2001). Also inter-catheter variations due to fluctuations in cerebral perfusion pressure and temperature have been reported (Galea et al., 2014). No direct in vivo comparison of different catheter sizes in MD sampling for analysis of chemokines or cytokines in TBI patients has previously been reported, although the impact of using different perfusion fluids has been investigated (Giorgi-Coll et al., 2020).

With these aspects in mind, it is difficult to perceive which inflammatory mediators are decisive for the evolution of secondary inflammatory brain damage in TBI. Previous studies report quenching of cytokine/chemokine release between 4 and 6 days after trauma, a notion that partially contradicts results from post-mortem and blood analysis showing signs of a persistent, long-term inflammation after TBI (Chaban et al., 2020; Frugier et al., 2010; Vedantam et al., 2021).

Therefore, the aim of the present study was to validate a large pore (300 kDa MW cut-off) MD membrane and to investigate if the cytokine levels differ based on location in the brain and if and how the patterns change in relation to secondary events such as periods of uncontrollable intracranial pressure (ICP) elevations. We also aimed at extending sampling time beyond 100 h after the trauma. Besides the necessity to pinpoint the qualitative and quantitative release of cytokines in TBI for more detailed unveiling of mechanisms, inflammatory mediators in TBI could also be used for monitoring in trials using anti-inflammatory drugs.

2. Materials and methods

2.1. Ethics

All research was conducted in accordance with the ethical standards given in the Helsinki Declaration of 1975, as revised in 2008. Ethical permission was granted by the regional ethical review board of Lund University, LU, Sweden (decision number LU-2017/469). As the use of microdialysis is clinical routine, the ethical permit covered the analysis of patients not included in clinical routine, eg cytokines and chemokines. Since the included TBI patients could not themselves consent to the study, a written informed consent was obtained from the patient's closest relative.

2.2. Clinical Aspects/patients

The study recruited 7 patients with isolated severe TBI, a Computed Tomography (CT) scan consistent with TBI, and a post-resuscitation Glasgow coma score (GCS) of ≤8 (Grande et al., 2002). All patients displayed extensive primary injuries that included traumatic intracranial hemorrhage. All patients but one had secondary surgery due to intractable ICP. The patient that only had surgery once underwent a primary decompressive craniectomy and simultaneous evacuation of two traumatic contusions. Monitoring was performed using an intraparenchymal or intra-ventricular intracranial pressure (ICP) monitor and microdialysis was performed with 300 kDa membranes, inserted together with the ICP monitor, and also, in 4/7 patients, in a pericontusional area at the time of craniotomy.

ICP and mean arterial blood pressure (MAP) was measured continuously and recorded automatically in the intensive care software IntelliSpace Critical Care and anesthesia (ICCA Philips Healthcare, Suresnes, France), Cerebral perfusion pressure (CPP) was calculated (MAP- ICP) and recorded.

2.3. Treatment according to TBI algorithm

Treatment according to modified principles of Lund Concept was given to all patients (Grande et al., 2002). Focal mass lesions (extracerebral hematomas or contusions) with a significant mass effect were evacuated, unless the traumatic hematomas were deep-seated. According to the protocol, blood pressure was controlled with clonidine and metoprolol. Sedation was maintained with midazolam and,occasionally, with thiopental.

Cerebrospinal (CSF) drainage was allowed when basic NICU treatment could not control ICP. If CSF drainage could not control ICP, thiopental was added.

Finally, if ICP could not be controlled, a decompressive hemicraniectomy was performed.

Fever was managed with paracetamol, and invasive cooling when needed to keep core temperature ≤ 38.5.

2.4. Microdialysis

300 kDa molecular weight cutoff MD catheters (CMA 320, Stockholm, Sweden) were used in all patients. 7 patients received 2 MD catheters, one placed in seemingly non-traumatized areas near the ICP monitor, and one placed in peri-contusional areas. All MD catheters were perfused with central nervous system perfusion fluid (CMA Microdialysis AB, Solna, Sweden) at 0.3 μL/min using CMA 106 micro infusion pumps. All microdialysis vials were kept at approximately the same height as the MD pumps in order to avoid hydrostatic forces that could affect the recovered volumes. Microdialysis catheters were categorized according to their position in relation to an injured area using the first postoperative CT-scan available. All catheters were clearly visualized on a CT-scan and termed "A" if placed in "seemingly normal" tissue, i.e. brain parenchyma without visible edema and blood. If the catheter was placed in close proximity to edema or blood, it was pericontusional and named "B".

The locations of all monitoring devices were confirmed on CT scans.

MD vials were changed hourly in all catheters and analyzed on an ISCUS (CMA Microdialysis AB, Sweden) bedside analyzer according to clinical routine. Remaining contents of vials were stored on dry ice in a cooling bag until they were transported to a − 80 °C freezer daily.

2.5. Cytokine analysis

All samples were analyzed using a multiplex kit (MesoScale Discovery, USA) according to the manufacturers' instructions. Microdialysates from a 6-h time period were pooled immediately before analysis, in order to yield sufficiently large volumes for analysis which was 200 μl. Cytokine measurements were plotted in the middle of the 6-h time period, to represent a mean value for the given period.

The microdialysates were analyzed with Proinflammatory cytokine-1 V-plex kit and Chemokine V-plex kit (see Table 2).

Due change of standards values for the proinflammatory cytokine-1 Vplex kit, concentrations were recalculated after linear regression computations with a constant of 0.7357. Both kits contain IL-8 but gave different values and the final IL-8 value was taken from the proinflammatory cytokine-1 V-plex kit. Samples were analyzed in duplicate.

2.6. Statistics

Differences between catheters in seemingly non-traumatized and peri-contusional tissue were calculated with the non-parametric Mann Whitney *U* test. Slope and *p* values for regression analysis of change over time were calculated with linear regression. All computations and graphics were performed with the free software R (https://www.R-project.org/).

3. Results

3.1. Patient demographics

2/7 patients had an isolated TBI and were ≤ GCS 8 on admission and 3 out of 7 patients had a GCS < 6. Recovery after 6 months ranged from GOSE 3 to 7. All patients survived (see Table 1).

3.2. Duration of microdialysis monitoring

In 4/7 patients, both MD catheters worked during the entire monitoring time. MD monitoring for metabolites was initiated at a mean time of 51 h post trauma (range 9–350 h) in six of the seven patients. The patient in whom MD monitoring was initiated at 196 h post-injury, received the initial treatment at another hospital and additional neurointensive care at arrival in our department. In the present study, we intended to monitor patients beyond 5 days after trauma, which was feasible in all patients.

3.3. Definition of consistently elevated cytokines

Using the Mesoscale Multiplex assay, the following chemokines and cytokines were analyzed: MCP-1 (CCL2), MIP-1β (CCL4), eotaxin (CCL13), MCP-4 (CCL13), TARC (CCL17), MDC (CCL22), eotaxin-3 (CCL26), IL-8 (CXCL8), IP-10 (CXCL10), IL-1β, IL-2, IL-6, IL-10, IL-12p70, GM-CSF, IFNγ and TNF-α (Table 2). Cytokines/chemokines that were found to be consistently elevated, defined as values >1000 pg/mL, at more than one time-point e.g. IL-6, IL-8, IP-10, MCP-1 and MIP-1β were tentatively considered to be most important in the development of inflammation following TBI.

These were investigated further in relation to glycerol, lactate-pyruvate ratio, ICP and CPP. Four other chemokines; Eotaxin, eotaxin-3, TARC and MDC had concentrations between 100 and 1000 pg/mL with mean values over 100 pg/mL. A third group encompassing IL-1β, IL-10, GM-CSF, IFNγ and TNF-α displayed median values between 10 and 100 pg/mL while IL-2 and IL-12 had mean values below 10 pg/mL

Table 2
Summary of analyzed chemokines/cytokines with 300 kDa membranes.

Name/Group	CCname	Receptor	Target	MW (kDa)
Top range				
MCP-1	CCL2	CCR2,5	Mcyte[a]	13-15
MIP1-β	CCL4	CCR1,5	Mcyte	7.8
IL-8	CXCL8	CXCR1,2	NPhil[b]	8.4
IP-10	CXCL10	CXCR3	Mcyte, Tcell[c]	8,6
IL-6	–	IL6R	Misc.	21–26
Mid range				
Eotaxin	CCL11	CCR2,3,5	Ephil[d]	8.4
MCP-4	CCL13	CCR1,2,3	Ephil	8.6
TARC	CCL17	CCR4	Tcell	10.3
MDC	CCL22	CCR4	Tcell	8.1
Low range				
IFNγ	–	IFNGR	Misc.[e]	17
GMCSF	–	CSF2R	Misc.	14–35
IL1-β	–	IL1R	Misc.	31
TNFα	–	TNFR	Misc.	17.3
IL-10	–	Il-10R	Misc.	18
IL-2	–	IL-2RA	Misc.	15.5
IL-12	–	IL12RA	Misc.	75

[a] Monocyte.
[b] Neutrophil granulocyte.
[c] T lymphocyte.
[d] Eosinophil granulocyte.
[e] Target cells undefined in TBI or miscellaneous.

Table 1
Patient demographics.

Patient	A	B	C	D	E	F	G
Age (years)	46	22	42	32	61	44	42
Sex (Male/Female)	M	F	F	F	F	M	M
GCS[1]	5	4	8	5	8	7	7
Pupils (+dilated/−normal)	−/−	−/+	−/−	+/−	−/−	−/−	−/+
Trauma mechanism	fall	mv	fall	mv	fall	fall	fall
Neurosurgical Diagnosis EDH/SDH/ICH[2]	Y/Y/Y	N/N/Y	N/N/Y	Y/Y/Y	N/Y/Y	N/N/Y	N/Y/Y
Time trauma - 1:st surgery	12 h	3 h	16 h	24 h	2 h	6 h	6 h
Time trauma - 2:nd surgery	35 h	45 h	51 h	216 h	37 h	73 h	N/A
DC[3]	N	Y	N	N	N	Y	Y
Time – with MD in hours	(59–175)	(50–126)	(52–162)	(219–359)	(37–160)	(80–131)	(9–139)
Days in NICU[4]	17	7	9	9	7	11	16
GOSE[5]	3	3	5	7	3	6	5

[1] Glasgow Coma Scale.
[2] EDH – Epidural hematoma, SDH – Subdural hematoma, ICH - Traumatic intracranial hemorrhage.
[3] Decompressive craniectomy.
[4] NICU – Neurointensive Care Unit.
[5] GOSE – Glasgow Outcome Scale Extended.

(see Fig. 1).

Differences between catheters in peri-contusional and seemingly non-traumatized areas 4 of the 7 patients had consistently functioning catheters in damaged and non-damaged areas, i.e. peri-contusional or seemingly normal areas. In these patients, comparison of cytokine and chemokine values between peri-contusional tissue and non-traumatized tissue was possible. In the present study, only IL-6 levels differed significantly between the catheters, peri-contusional: 1467(22–2912) pg/mL versus non-traumatized: 429(314–544) pg/mL (median(IQR)), *p*-value = 0,000036. No significant differences between peri-contusional and non-traumatized areas of the chemokines IL-8, IP-10, MCP-1 and MIP-1β were observed. (Fig. 2).

No correlations between cytokine/chemokine secretion and ICP.

Using the modified Lund Concept algorithm, ICP could be controlled in all patients during monitoring time, and no secondary insults such as cerebral Ischemia or infarction were noted. As a result, we did not observe any major changes in ICP that could have mirrored changes in cytokine/chemokine levels.

3.4. Microdialysis metabolite pattern

The L/P ratio was defined as pathological when >25. A pathological L/P ratio was registered in patient B and C on the peri-contusional side, but also in patient D on the seemingly normal side. Glycerol was defined as pathological when >100 μmol/l (Belli et al., 2008). Glycerol was slightly elevated in patient A and pathological in patients C and D on the peri-contusional side, but also pathological in patient A on the non-traumatized side. Neither changes in glycerol nor L/P ratio covaried with the cytokine/chemokine secretion (Fig. 2).

3.5. Temporal pattern of cytokine/chemokine secretion

Slope and *p* values for each cytokine or chemokine were calculated for all 6 patients together. When all patients were included in the analysis no significant increases or decreases in chemokines/cytokines were observed. If the patient admitted at day 10 and monitored until day 14 was excluded from the analysis, IL-6, IP-10 and MCP-1 from the peri-contusional area were significantly decreased from 71 ± 5 h to 141 ± 14 h, whereas the slope for the non-traumatized area was not affected. (Fig. 3).

4. Discussion

The rationale for measuring cytokines from the cerebral extracellular space in TBI are multiple; firstly to verify that TBI induces tissue inflammation, secondly to classify the patterns of the inflammatory response and thirdly to assess whether the temporal or quantitative inflammatory profile influences, or is influenced by, alterations of other factors induced or observed in secondary injury. Finally, assessment of inflammation in TBI can be used to monitor interventions aimed at modulating outcome by targeting specific components of the inflammatory response. In the present study, we for the first time report

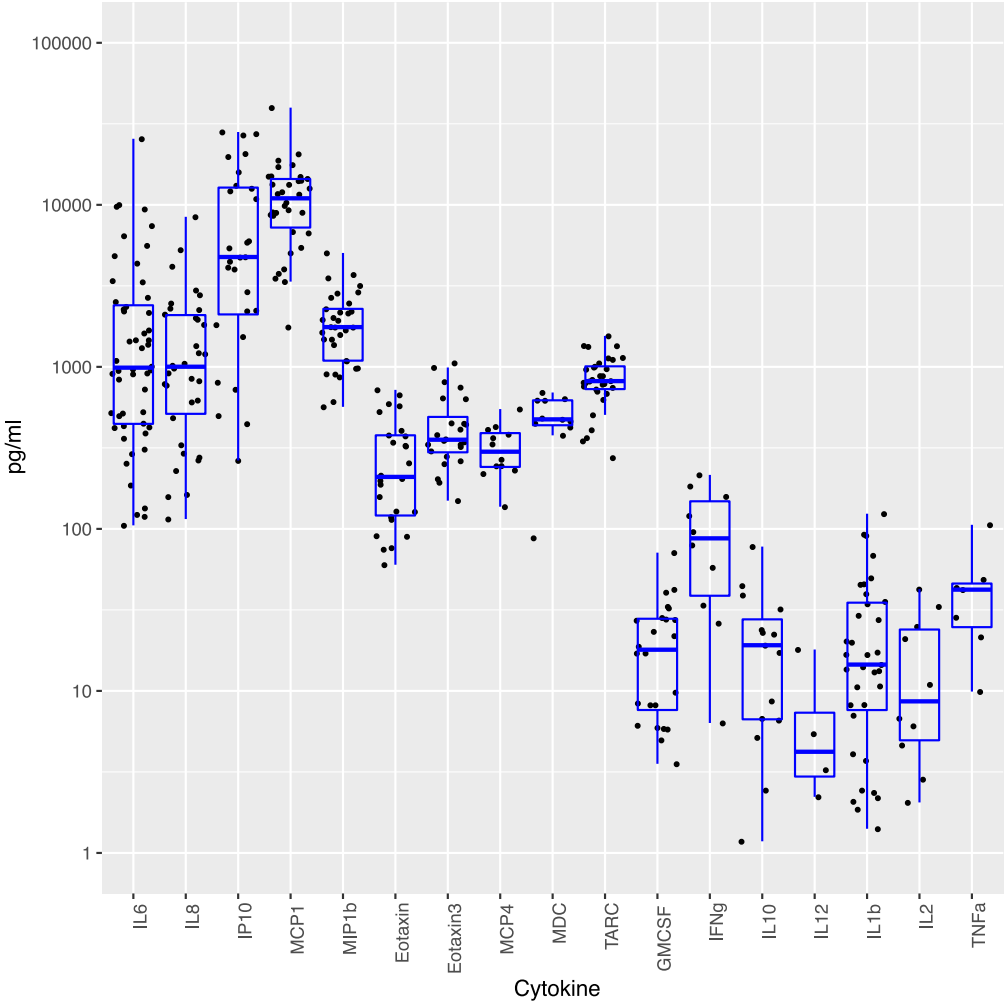

Fig. 1. Analyzed cytokines and chemokines for all patients depicted on a logarithmic scale.

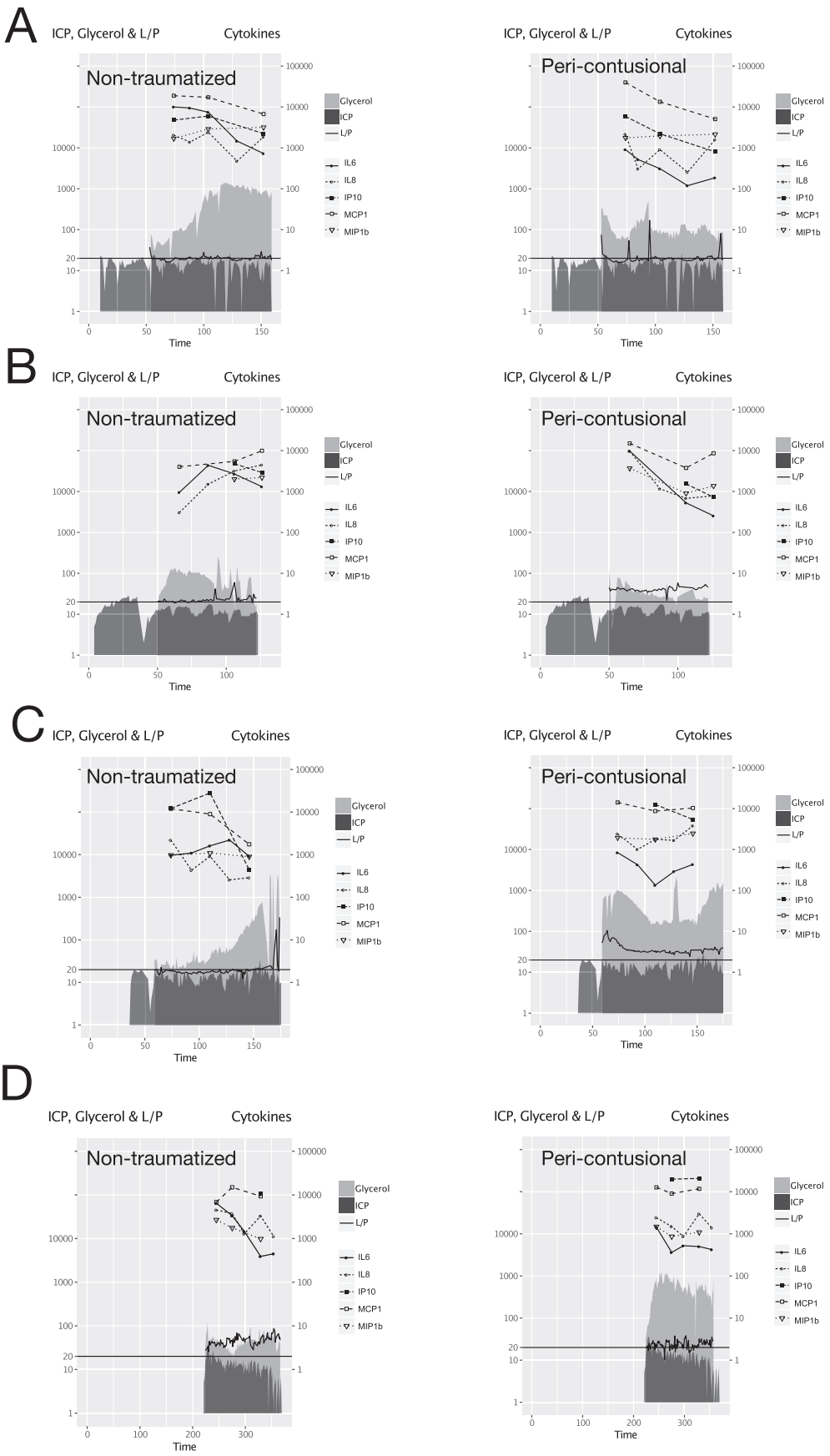

Fig. 2. Patient monitoring data is displayed as Glycerol (gray-filled line), ICP (black filled line), and MD Lactate/Pyruvate ratio (black line). Critical ICP threshold according to the TBI algorithm (20 mmHg) (A) patient 3 (B) patient 5, (C) patient 6 and (D) patient 7.

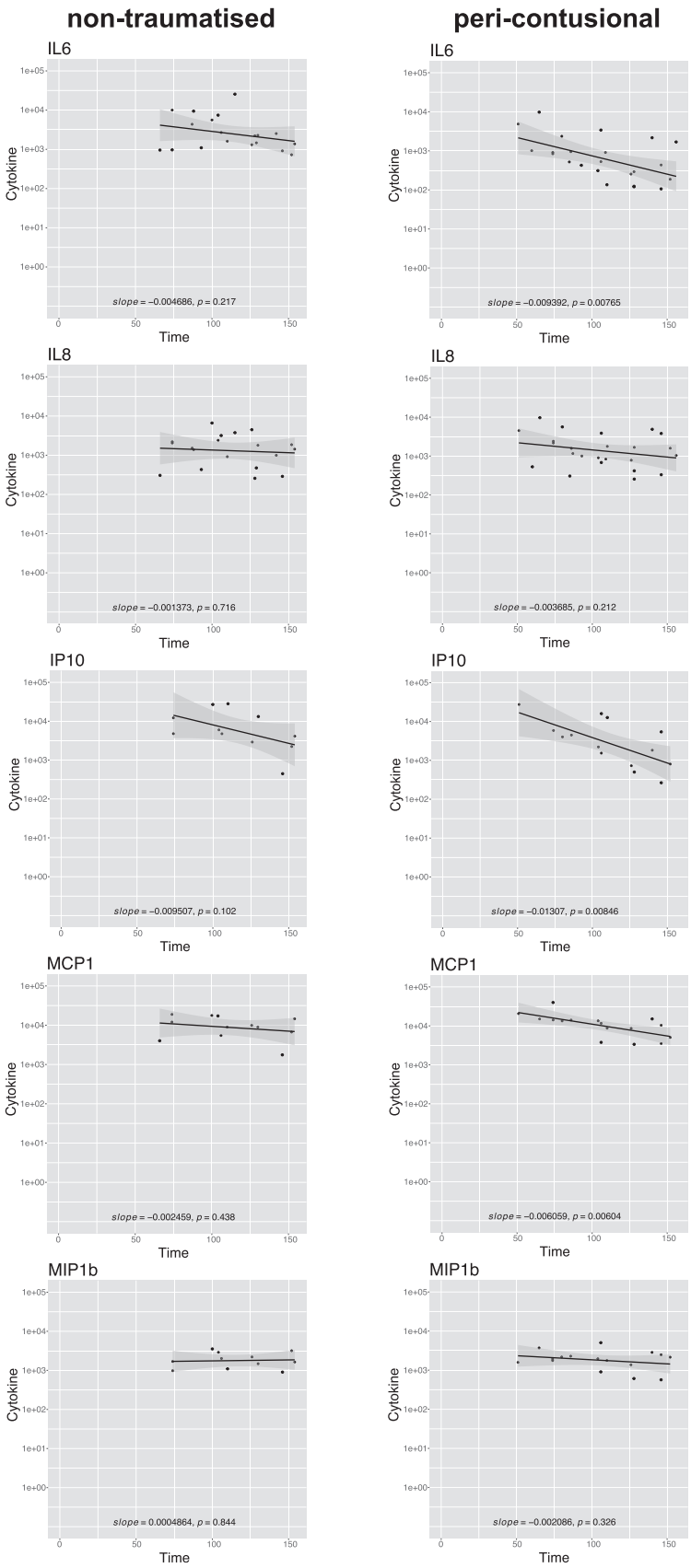

Fig. 3. Slope values denoting changes in pico-grams per hour. For non-traumatized areas, no significant increases or decreases chemokine/cytokine levels were observed. For peri-contusional areas, a significant decrease was observed for IL6, IP10 and MCP1.

measurement of cytokines and chemokines in TBI beyond 5 days after trauma and from seemingly non-traumatized and peri-contusional areas by cerebral MD using 300 kDa membranes,

4.1. Definition of consistently elevated inflammatory mediators

Due to the availability of multiplex kits, most studies have reported a heterogenous range of cytokines and chemokines, where the choice has depended more on kit content than on study hypothesis.

In the present case series, we could discern four categories of chemokines /cytokines based on quantitative levels defining a high range group (IL-6, IL-8, IP-10, MCP-1 and MIP-1β) a mid-range group (Eotaxin, Eotaxin-3, MDC, MCP-4 and TARC), a low range group(IL-1β, IL-10, GM-CSF, IFNγ and TNF-α) and a very low range group (IL-2, IL-12). Both the high range and the mid-range group have been reported in previous MD studies using 100 kDa catheters in TBI (Bartek Jr. et al., 2019; Giorgi-Coll et al., 2017; Perez-Barcena et al., 2011). However, there is the possibility that certain mediators have a biological activity even at lower concentrations while others have their effects at higher concentrations, either directly or indirectly (Thelin et al., 2018). Using 300 kDa membranes, higher concentrations of all inflammatory mediators were observed compared to previous studies using 100 kDa membranes (see Table 2). Given that recovery of chemokines/cytokines is only partial, at best, the actual levels in the brain are likely even higher. This indicates that inflammation after TBI resembles a local cytokine storm of similar magnitude as those induced by CNS infections or cytokine release syndromes (Table 2), but confined to the CNS (Fajgenbaum and June, 2020; Gupta et al., 2017).

4.2. Patients

The subtype of TBI that induces the most vigorous tissue inflammation in humans is unknown but contusional injuries are most likely to do so (Woodcock and Morganti-Kossmann, 2013). Secondary events such as ischemia or hemorrhage, but plausibly the extent of surgical and intensive care treatments, could additionally influence the evolution of tissue inflammation. In the present study, traumatic contusions were found in all patients and in all patients cytokine and chemokine values over 1000 pg/mL could be detected at some time points thus indicating extensive tissue damage.

4.3. Temporal changes

Earlier reports have found significantly increased plasma levels of IL-6, IL-8 and MCP-1 several months after severe and mild TBI and these partially correlate with outcome (Chaban et al., 2020; Rusiecki et al., 2020; Sun et al., 2019). In previous studies, cytokine/chemokine expression reach peak values within 24–48 h after MD-catheter implantation (Giorgi-Coll et al., 2017; Perez-Barcena et al., 2011; Winter et al., 2002). In our material, MD samples have been sampled from 9 to 359 h after the primary insult with only minor declines in cytokine/chemokine expression, and thus in contradiction to previous results, as we did not observe any clear decline of the levels of inflammatory mediators during the sampling time, except for a partial decrease in the peri-contusional area for IL-6, MCP1 and IP10. The reasons for this discrepancy could be multiple; different injuries in our study population, different therapeutic interventions and smaller pores of the 100 kDa catheters in previous studies. The reported early peaks and fast decline of cytokines/chemokines may tentatively be due to loss of recovery from catheters, either by clogging or fibrosis in the vicinity of the membrane. We present our data with the zero-time point at the time of the trauma. Most patients were referred from other hospitals and therefore our zero-time point may differ from other studies where the zero-time point is presented as the time when the patient received the MD catheter, whereas this time point may well be 24 h after the trauma.Different methods to increase in vitro recovery in microdialysis studies have included antibody coated microspheres, nano-gold particles, dextran, albumin, plasma and hydroxyethyl starch (HES) but none have addressed the possibility of late partial or total blocking of catheters (Ao et al., 2004; Giorgi-Coll et al., 2017; Giorgi-Coll et al., 2020; Khan et al., 2015).

The levels of some chemokines/cytokines, mainly IL-6, IL-8 and IL-1β are extremely sensitive to tissue damage even as minute as that caused by inserting the MD catheter (Carson et al., 2015). Bouras et al. implanted 31 intracerebral MD catheters in 12 patients with epilepsy undergoing invasive EEG monitoring (Bouras et al., 2021). Surprisingly, this study found that the cytokines IL-1β, IL-6, IL-8, peaked within 24 h and thereafter declined to reach low steady state levels within 72–120 h in non-gliotic areas. In gliotic areas, levels of IL-6, IL-8 and TNF-α showed the same pattern but IL-1β was increased. This implies that IL-6, IL-8 and TNF-α were induced by the introduction of the MD catheter while IL-1β could represent true tissue inflammation. Taken together, these results do not exclude the possibility that cytokine levels in TBI MD studies initially may reflect catheter inflicted tissue damage. However, because Bouras et al. also used 100 kDa catheters, the early decline of cytokine levels reported could partially be caused by catheter clogging.

4.4. Differences between catheters in peri-lesional and seemingly non-traumatized areas

Despite that previous studies have reported difference for metabolites in peri-contusional and seemingly non-traumatized areas, no conclusive data exist for cytokine/chemokine expression in TBI. Helmy et al. (Helmy et al., 2011) used two MD catheters, both in non-traumatized areas for comparison of relative recovery of cytokines/chemokines using two different perfusion fluids. Mellergard et al. (Mellergard et al., 2008). used two MD catheters placed in non-traumatized area and peri-lesional, but only used the catheter which yielded the highest concentrations of glycerol during the first 12–18 h after insertion for further analysis. In the present study only IL-6 levels differed between peri-lesional and non-traumatized areas. This could reflect that IL-6 is mainly produced by astrocytes and neurons while the chemokines may be produced by infiltrating cells. However, this does not exclude the possibility that there could be an initial difference before our first samplings at 50 h. Our findings could thus depend on either rapid spread of inflammatory mediators in brain parenchyma or that the inflammatory response is evoked simultaneously in multiple areas.

4.5. Covariation with ICP and metabolites

We could not observe any relation between cytokine/chemokine expression and potential secondary insults as measured by metabolites or minute ICP changes. This is consistent with the finding of a previous study that also failed to show any correlation between clinical events and cytokine/chemokine levels (Perez-Barcena et al., 2011). This may not be surprising since the raised levels of chemokines and cytokine were relatively unchanged during sampling time. There could still be a covariation at early time points prior to our first samples. It is also possible that though the inflammatory cascade induces cerebral edema over a longer time, other mechanisms as perfusion related events, regulate minute changes in ICP.

4.6. Influence of treatment algorithm

Several drugs commonly used in different algorithms for severe TBI treatment including propofol, midazolam, clonidine, metoprolol and barbiturates may all influence the cytokine and chemokine expression. The Lund Concept algorithm used in the present study includes clonidine and metoprolol, which may have had an effect which cannot be identified in this study, since all patients were treated with clonidine and metoprolol. Which both can modulate the chemokine and cytokine levels (Schroeppel et al., 2010). Also, midazolam, pentothal and

propofol have been shown to modulate cytokine/chemokine expression (Cruz et al., 2017; Kallioinen et al., 2019; Rossano et al., 1992).

4.7. Importance of pore size

Considering different sampling time and assays it is still obvious that the 300 kDa membrane gives a higher yield than those reported for 100 kDa membranes (see Tables 2 and 3). The recovered levels of the smaller size chemokines could be higher than for the larger size cytokines. Unfortunately, there are no relevant studies comparing MD with direct sampling from brain tissue for chemokines and cytokines.

4.8. Mechanisms of cytokine/chemokine influx

The sequence of cellular infiltration in experimental TBI encompasses early neutrophil influx followed by monocytes/macrophages and plausibly a later arrival of T- and B-cells (Alam et al., 2020; Needham et al., 2019). Simultaneously, CNS intrinsic cells as microglia, resident CNS macrophages, astroglia and neurons are activated in a complex and reciprocal network (Jacob Rodrigues et al., 2020). Chemokine receptors also have a skewed immune cell distribution where CCR2 is mainly located on monocytes/macrophages, CCR3 on eosinophils and basophils, CCR4 on microglia and CXCR2 on neutrophils and CXCR3 on T-cells(Gyoneva and Ransohoff, 2015). In the high and mid-range groups of detected chemokines/cytokines, all have a documented chemotactic effect on neutrophils or monocytes/macrophages, even the only non-chemokine IL-6 (Clahsen and Schaper, 2008). There is a certain redundancy of chemokines and their receptors at least at resting conditions where some share the same receptors as Eotaxin/MCP-4 (CCR3) Eotaxin-3/TARC/MDC (CCR4). This could mean that some of the measured molecules are mostly ¨noise¨ in the local cytokine/chemokine storm. Although all chemokines have been shown to act as chemoattractants during varying conditions, it is possible that some are more important, as has been proposed for MCP-1 for monocyte attraction and IL-8 for neutrophil attraction(Gyoneva and Ransohoff, 2015). These are both included in the group of high range chemokines and cytokines.

Despite the fact that certain chemokines and cytokines have been associated with worse outcome (MCP-1, IL-6), no persistent pattern has yet been put forward, emphasizing the notion that measurements of single mediators have no clear correlation to other parameters (Simon et al., 2017). The low range and very low range group consists of inflammatory cytokines that were not detected in all patients. It is possible that some of the cytokines were present, but at concentrations limited for detection by this assay (0,5–1,5 pg/mL). We do recognize that these cytokines may play an important role, even at very low concentrations. Some are classified as pro-inflammatory cytokines (IL-1β, TNF-α), others have been designated as anti-inflammatory (IL-10) while the remaining have an undecided role (GM-CSF, IL-12, IL-2). As they are not detected in all patients they might not only be the result of tissue damage but of events such as an ongoing infection, actual therapy or autoimmunity.

Besides functioning as chemo-attractants for infiltrating inflammatory cells, the high-range and mid-range chemokines could also have other effects in TBI. Both MCP-1, IP10, IL-8 and IL-6 have been implicated in neuronal damage and edema formation by signaling to CNS resident cells as microglia (Wang and Colonna, 2019) astrocytes (Sun et al., 2017) and neurons (Du et al., 2018).

As discussed, the levels of chemokines/cytokines after TBI might not mimic their impact in the evolution of secondary damage in TBI. In this respect both TNF-α and IL-1β have been proposed to initiate the release of other pro-inflammatory cytokines in an experimental in vitro model (Thelin et al., 2018).

5. Conclusions

The major limitation of this study, is that the results are based on analysis of 7 patients. However, the results show potent and persistent pan-cerebral cytokine and chemokine levels far beyond the first 5 days after the trauma with no signs of decline in patients with severe TBI.

A high range group, of mainly chemokines, was identified but only IL-6 from this group showed a gradient from traumatized to non-traumatized tissue when dual catheters were used. The results implicate that severe TBI induces a long standing cerebral neuroinflammation.

Table 3
Comparison of chemokine/cytokine values in TBI and cytokine release syndrome.

Ch/Ckin	TBI-EC-Md100[a]	TBI-EC-Md300[b]	CRS-blood[c]	TBI-blood[d]
MCP-1	2500	11,000	60	<300
IP-10	2834	4000	2000	<200
MIP1-β	38	1500	60	<200
IL-6	570	1000	3200	<400
IL-8	339	1000	575	11
TNFα	1	40	52	<5
IFNγ	7	80	3722	NA
IL1-β	6	20	5	<1

[a] Cytokine/chemokine values from extracellular fluid /microdialysis with 100 kDa cut off membranes-ref 20,[30] (Helmy et al., 2011; Perez-Barcena et al., 2011).
[b] Cytokine/chemokine values from extracellular fluid /microdialysis with 300 kDa cut off membranes-present study.
[c] Cytokine/chemokine values from blood in patients with cytokine releases syndrome aka cytokine storm-ref [18] (Gupta et al., 2020).
[d] Cytokine values from blood in TBI patients-ref [8,13,36] (Chaban et al., 2020, Frugier et al., 2010, Smith et al., 2013).

Abbreviations

Abbreviations

TBI	Traumatic Brain Injury
MD	Microdialysis
ICP	Intracranial pressure
kDa	Kilo Dalton
DAMPs	Disease associated molecular patterns
CSF	Cerebrospinal fluid
MW	Molecular weight

Data availability

Data will be made available on request.

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