Published in final edited form as:

Pediatr Crit Care Med. 2015 July ; 16(6): 549–557. doi:10.1097/PCC.0000000000000400.

Cerebrospinal Fluid Markers of Macrophage and Lymphocyte Activation after Traumatic Brain Injury in Children

Elizabeth Newell, MD^{1,*}, David K. Shellington, MD^{2,*}, Dennis W. Simon, MD³, Michael J. Bell, MD^{3,4}, Patrick M. Kochanek, MD, FCCM^{3,5,6}, Keri Feldman, BS³, Hülya Bayır, MD^{3,7}, Rajesh K. Aneja, MD^{3,5}, Joseph A. Carcillo, MD, FCCM³, and Robert S. B. Clark, MD^{3,5,6,8}

¹Department of Pediatrics, University of Iowa

²Department of Pediatrics, University of California San Diego

³Department of Critical Care Medicine, University of Pittsburgh School of Medicine

⁴Department of Neurological Surgery, University of Pittsburgh School of Medicine

⁵Department of Pediatrics, University of Pittsburgh School of Medicine

⁶Department of Anesthesiology, University of Pittsburgh School of Medicine

⁷Department of Environmental and Occupational Health, University of Pittsburgh School of Medicine

⁸Clinical and Translational Science Institute, University of Pittsburgh School of Medicine; The Children's Hospital of Pittsburgh of UPMC, and the Safar Center for Resuscitation Research, University of Pittsburgh School of Medicine

Abstract

Objective—The magnitude and role of the cellular immune response following pediatric traumatic brain injury (TBI) remains unknown. We tested the hypothesis that macrophage/microglia and T-cell activation occurs following pediatric TBI by measuring cerebrospinal fluid (CSF) levels of sCD163 and ferritin, and sIL-2R α , respectively, and determined whether these biomarkers were associated with relevant clinical variables and outcome.

Design—Retrospective analysis of samples from an established, single-center CSF bank.

Setting—Pediatric Intensive Care Unit (PICU) in a tertiary Children's Hospital

Patients—Sixty-six pediatric patients after severe TBI (Glasgow coma scale score [GCS]<8) age 1 mo-16 y and 17 control patients age 1 mo-14 y.

Measurements and Main Results—CSF levels of sCD163, ferritin, and sIL-2R α were determined by ELISA at 2 time points ($t_1=17\pm10$, $t_2=72\pm15$ h) for each TBI patient. CSF sCD163, ferritin, and sIL2R α levels after TBI were compared with controls and analyzed for associations

with age, patient sex, initial GCS, diagnosis of abusive head trauma (AHT), the presence of hemorrhage on computerized tomography scan, and Glasgow outcome scale score (GOS).

CSF sCD163 was increased in TBI patients at t_2 vs. t_1 and controls (95.4[21.8–134.0] vs. 31.0[5.7–77.7] and 27.8[19.1–43.1] ng/ml, respectively; median[IQ]; $P<0.05$). CSF ferritin was increased in TBI patients at t_2 and t_1 vs. controls (8.3[7.5–19.8] and 8.9[7.5–26.7] vs. [7.5[0.0–0.0] ng/ml, respectively; $P<0.05$). CSF sIL-2R α in TBI patients at t_2 and t_1 were not different vs. controls. Multivariate regression revealed associations between high ferritin and age ≤ 4 y, lower GCS, AHT, and unfavorable GOS.

Conclusions—Children with TBI demonstrate evidence for macrophage activation after TBI, and in terms of CSF ferritin, this appears more prominent with young age, initial injury severity, AHT, and unfavorable outcome. Further study is needed to determine whether biomarkers of macrophage activation may be used to discriminate between aberrant and adaptive immune responses, and whether inflammation represents a therapeutic target after TBI.

Keywords

cd163; ferritin; head injury; interleukin-2 receptor α ; macrophage activation syndrome; microglia

Traumatic brain injury is the most common cause of death and disability in children with nearly 500,000 children suffering from TBI each year (1). Unfortunately, specific therapies are lacking with existing treatment primarily being supportive. Given that the tools to reverse primary tissue injury after TBI are currently lacking, significant effort is being placed on identifying means of manipulating secondary injury pathways that follow TBI and contribute to ongoing cellular dysfunction and death. Inflammation is one such secondary process that may represent a therapeutic target following TBI. Inflammation following experimental TBI has been well documented and is mediated by both CNS immune cells as well as those of the peripheral immune system following disruption of the blood-brain barrier (BBB)(2). There are also reports identifying cellular inflammation after TBI in adult patients (3, 4). There are caveats in regard to manipulating neuroinflammation; however, as it has been shown to have both beneficial and detrimental roles following TBI. These divergent roles likely depend on timing (5, 6), severity (7), and type of injury (8), as well as on age (9), genetics (10), and other factors. As such, strategies will be required that harness the beneficial effects such as repair, trophic factor release, and debridement, while minimizing undesirable effects such as edema and supraphysiologic inflammation. (11). Identifying markers of an aberrant or supraphysiologic inflammatory state would thus be useful in attempting to strategically manipulate the neuroinflammatory response after TBI.

An extreme example of an aberrant inflammatory state is hemophagocytic lymphohistiocytosis (HLH), a condition of immune dysregulation resulting in hyperinflammation due to uncontrolled macrophage and T-cell activation. A case definition of HLH requires diagnostic criteria identifying macrophage and T-cell activation, including measurement of ferritin and soluble interleukin-2 receptor α (sIL-2R α), respectively (12). More recently soluble cluster of differentiation 163 (sCD163) has also been suggested to be a marker of macrophage activation (13). Neurological involvement is common in HLH, occurring in 30–50% of cases at presentation (12), and includes neuroinflammation,

necrosis, white matter abnormalities, and hemorrhage (14). Coincidentally, Rooms et al. (15) reported a series of three children with brain injuries suspected to be caused by abusive head trauma (AHT) that met diagnostic laboratory criteria for HLH raising concerns that AHT may lead to systemic activation of the immune system, or that systemic macrophage or resident microglial activation could cause intracranial findings that could be misconstrued as findings of child abuse (15). While the pathophysiology of CNS involvement in HLH is not completely understood, it likely is in part due to local uncontrolled macrophage and T-cell activation.

These studies raise the question of whether excessive CNS immune activation could be triggered by an inciting event such as trauma, and whether this could be identified by similar markers of macrophage/microglial and T-cell activation. Accordingly, the purpose of this study was to investigate the presence of markers of macrophage/microglial and T-cell activation in pediatric TBI, as characterized by elevations in cerebrospinal fluid (CSF) sCD163, ferritin, and sIL-2R α . While previous studies have looked at various markers of inflammation following pediatric TBI (16–21), this is the first study looking specifically for the presence of markers of macrophage and T-cell activation. These findings may represent a means for identification of patients likely to respond to strategies targeting cellular inflammation, and/or a means for therapeutic drug monitoring for future clinical trials targeting inflammation after TBI.

MATERIALS & METHODS

Patients

The Institutional Review Board of the University of Pittsburgh granted approval for CSF collection from patients with severe TBI. CSF from children with severe TBI, defined as a Glasgow coma scale score (GCS) ≤ 8 , either initially or after deterioration, collected from patients admitted to the pediatric intensive care unit (PICU) at the Children's Hospital of Pittsburgh from 2000 to 2009 were used for this study. Lumbar CSF previously obtained from children undergoing lumbar puncture for the evaluation of possible infection, but ultimately proven to be infection free, was used as control samples for this analysis.

The clinical care for children with severe TBI at our institution has been standardized and is based on current guidelines (22). This includes placement of an external ventricular drain (EVD) for measurement of intracranial pressure (ICP) and CSF diversion. CSF was continuously drained by gravity into a reservoir at the bedside positioned 3–10 cm above the mid-brain. CSF was collected using sterile technique every 12–24 h for up to 7 d after TBI and stored at -80°C for later analysis. A subset of children received early hypothermia ($32-33^{\circ}\text{C}$ for 48 h followed by slow rewarming) as part of an ongoing clinical trial (23). The following clinical variables were recorded and catalogued: age at the time of injury, patient sex, initial GCS (iGCS), presence of hemorrhage on head computerized tomography (CT) scan, diagnosis of abusive head trauma (AHT) by the hospital's Child Advocacy Center, inclusion and group assignment (if applicable) in the therapeutic hypothermia trial, and Glasgow outcome scale score (GOS) at 6 mo after injury. Lumbar CSF from control patients without laboratory evidence of infection was also stored at -80°C . Demographic data such

as age and patient sex was often restricted in these patients; however, if available it was also recorded.

Quantification of CSF CD163, ferritin, and IL-2R α

CSF collected from two time points was assayed for TBI patients. Efforts were made to maintain consistency in the time points used; however, there was some variability due to different times at which CSF was collected from individual patients. The average ($\pm$ SD) collection time for $t_1 = 17 \pm 10$ h and for $t_2 = 72 \pm 15$ h. CSF levels of sCD163, sIL-2R α , and ferritin were determined using commercially available ELISA kits (R&D Systems DC1630 for sCD163, DR2A00 for sIL-2R α ; and Alpha Diagnostic International 1810 for ferritin). Optimal dilutions were determined and ELISAs were performed according to the manufacturer's instructions. Lower limits of detection (LLD) defined by the manufacturer for sCD163, sIL-2R α , and ferritin were 0.18 ng/ml, 10.0 pg/ml, and 7.5 ng/ml, respectively. Data points falling below the minimum range of detection were assigned the value of the LLD for the respective ELISA.

Statistical Analysis

Data are presented as mean $\pm$ SEM or median [interquartile range; IQ] where appropriate. Differences between t_1 , t_2 , mean, and peak CSF CD163, IL-2R α , and ferritin from TBI patients and control patients were determined using ANOVA on ranks with Dunn's *post hoc* test, as the data were not normally distributed. Comparisons between individual biomarkers were made using Spearman rank correlations. Univariate analyses comparing CSF CD163, IL-2R α , and ferritin within TBI clinical subgroups were performed using Mann-Whitney rank sum test. Since normative values for CSF sCD163, sIL-2R α , and ferritin after TBI in children are not available, high levels were defined as a CSF level $>$ median value for all respective TBI samples. Clinical variables were dichotomized as follows based on published criteria (16–21): age at the time of injury ≤ 4 y vs. > 4 y, iGCS 3–4 vs. 5–15, presence vs. absence of hemorrhage on head CT, diagnosis of AHT vs. accidental injury, treatment with hypothermia vs. normothermia or no inclusion in hypothermia trial, and favorable GOS (4, 5) vs. unfavorable GOS (1–3). Multivariate logistic regression was used to identify associations between high CSF CD163, IL-2R α , and ferritin levels and dichotomized clinical variables including outcome. SigmaPlot 11.0 (Systat Software, Inc., San Jose, CA) or STATA 12.0 (StataCorp, LP, College Station, TX) software were used. A P -value < 0.05 was regarded as significant.

RESULTS

Demographic data for the control and pediatric TBI patients are shown in Table 1. For the cohort where sCD163 and sIL-2R α were assayed there were 66 patients. In 59 of these patients, ferritin was also assayed. The mechanism of injury was accidental in 49 patients (74.2%) and AHT from child abuse in 17 (25.8%) patients. The overall group mortality rate was 12.1%. Thirty patients (45%) with severe TBI were concurrently enrolled into various trials to assess the effectiveness of hypothermia, with 15 of these patients randomized to therapeutic hypothermia.

CSF sCD163, sIL-2R α , and ferritin in TBI vs. control patients

Figure 1A shows CSF sCD163 levels from control and TBI patients. CSF sCD163 was increased in TBI patients at t_2 vs. t_1 and controls (95.4[21.8–134.0] vs. 31.0[5.7–77.7] and 27.8[19.1–43.1] ng/ml, respectively; $P < 0.05$), with sCD163 levels maximal during t_2 . For individual TBI patients peak (103.0[2.0–357.0] ng/ml), but not mean (68.0[1.5–217.6] ng/ml) sCD163 levels were also increased vs. control patients ($P < 0.05$). The median sCD163 level for all TBI patients used as a cutoff for “high” vs. “low” level was 46.9 ng/ml.

Figure 1B shows CSF sIL-2R α levels from control and TBI patients. CSF sIL-2R α was not different in TBI patients at t_2 or t_1 vs. controls (43.8[19.9–85.6], 47.9[18.2–108.1], vs. 36.9[22.1–76.7] pg/ml, respectively; $P > 0.05$). For individual TBI patients neither peak (57.3[7.4–379.2] pg/ml) nor mean (50.1[7.4–322.6] pg/ml) sIL-2R α levels were different vs. control patients ($P > 0.05$). The median sIL-2R α level for all TBI patients used as a cutoff for “high” vs. “low” level was 46.0 pg/ml.

Figure 1C shows CSF ferritin levels from control and TBI patients. CSF ferritin was increased in TBI patients at t_2 and t_1 vs. controls (8.3[7.5–19.8] and 8.9[7.5–26.7] vs. [7.5[0–0] ng/ml, respectively; $P < 0.05$; all 9 controls below LLD), with ferritin levels maximal during t_1 . For individual TBI patients peak (14.7[7.5–333.6] ng/ml) and mean (11.1[7.5–201.9] ng/ml) ferritin levels were also increased vs. control patients ($P < 0.05$). The median ferritin level for all TBI patients used as a cutoff for “high” vs. “low” level was 8.3 ng/ml.

As sCD163, sIL-2R α , and ferritin levels were assayed from CSF samples from the same patients, we analyzed the relationship of each biomarker to one another. Figure 2 shows that all three biomarkers are highly correlated with each other after TBI within both time points analyzed.

Univariate associations between CSF sCD163, sIL-2R α , and ferritin and clinical variables

Tables 2–4 show CSF sCD163, sIL-2R α , and ferritin levels in TBI patient subgroups dichotomized by age at the time of injury 4 y vs. > 4 y, iGCS 3–4 vs. 5–15, presence vs. absence of hemorrhage on head CT, diagnosis of AHT vs. accidental injury, and unfavorable GOS (1–3) vs. favorable GOS (4–5). There were no differences in CSF sCD163 within any of the subgroups analyzed (Table 2). CSF sIL-2R α was higher in patients 4 vs. > 4 y by univariate analysis (116.8[44.7–178.3] vs. 43.8[18.7–97.0] pg/ml, respectively; $P < 0.001$; Table 3). CSF ferritin was higher in patients 4 vs. > 4 y (36.5[14.7–56.0] vs. 9.1[7.5–21.0] ng/ml, respectively; $P = 0.001$); with a GCS of 3–4 vs. 5–15 (25.2[17.8–49.2] vs. 10.5[7.5–38.2] ng/ml, respectively; $P = 0.024$); and assigned unfavorable outcome with a GOS of 1–3 vs. favorable outcome with a GOS of 4–5 (27.9[12.6–68.0] vs. 10.1[7.5–27.4] ng/ml, respectively; $P = 0.014$; Table 4).

Given the relevance of intracerebral hemorrhage when evaluating macrophage and lymphocyte biomarkers in the CSF, CSF values for each biomarker separated by subgroups with or without intracerebral hemorrhage (ICH) on head CT are also shown in Tables 2–4. There were no differences between groups in any of the three biomarkers assayed ($P >$

0.05). There were also no differences between normothermia vs. hypothermia groups in CSF levels of sCD163, sIL-2R α , and ferritin ($P > 0.05$; Tables 2–4).

Clinical variables independently associated with CSF sCD163, sIL-2R α , and ferritin

Multivariate logistic regression models were constructed to detect independent associations between CSF biomarkers and clinical variables. Age and mechanism of injury were included in the statistical models given known influence on outcome after TBI. Patient sex was included in the statistical model given a trend towards higher levels for all three biomarkers in female vs. male patients. Initial GCS was included in the model to adjust for injury severity. Presence or absence of ICH was included based on the rationale discussed above. GOS assigned at 6 months was included to determine if biomarkers were associated with outcome. For each biomarker, high vs. low peak levels (based on median value for all TBI patients) were used for logistic regression. There were no independent associations between high sCD163 or high sIL-2R α and any of the clinical variables analyzed (not shown). Multivariate logistic regression revealed that high CSF ferritin was associated with age 4 y, lower initial GCS, AHT, and unfavorable GOS (Table 5). Note that no patient in the subgroup with an iGCS of 3–4 had a low CSF ferritin level.

DISCUSSION

We report that markers of macrophage/microglia activation (sCD163 and ferritin) but not a marker of lymphocyte activation (sIL-2R α), are increased in CSF following pediatric TBI. CSF ferritin was noted to be higher during the first time point assessed (17 ± 10 h), whereas sCD163 was higher during the second time point (72 ± 15 h). This could be consistent with early release of ferritin from damaged and activated microglia (24) or oligodendrocytes (25), with a later increase in sCD163 from systemic, infiltrating macrophages or perivascular microglia scavenging hemoglobin (26). A clinical study has shown early increases (<12 h) in ferritin within contusions after TBI in humans (27), whereas experimental data suggest a more delayed increase (72 – 96 h) in CD163-positive macrophages/microglia after TBI in rats (28). While CSF sIL-2R α was not different compared with CSF from control patients, it was strongly correlated with biomarkers of macrophage/microglial activation, suggesting the possibility of coordinated activation of macrophages/microglia and T-cells in the CNS following pediatric TBI. It is also possible that timing of CSF collection occurred prior to alterations in sIL-2R α . Serum elevations of these biomarkers occur together in the hyper-immune activation that occurs in HLH/macrophage activation syndrome in humans. Our data suggest some similarities but also differences in the inflammatory response after TBI compared with the prototypical hyper-immune response seen in patients with HLH.

Because serum ferritin is a recognized acute phase reactant that is increased with inflammatory and malignant conditions, CSF ferritin has been studied as a potential biomarker for a variety of neurologic diseases. It is known that CSF ferritin is elevated in subarachnoid hemorrhage, bacterial meningitis, multiple sclerosis, and different CNS malignancies (27, 29, 30). Primary mechanical damage after TBI would lead to non-selective release of intracellular ferritin, known to be present in neurons, astrocytes, and oligodendrocytes, in addition to microglia (24–27, 31, 32). However, evidence also points to

active secretion by macrophages/microglia as an additional source of CSF ferritin. Keir et al. evaluated CSF ferritin levels in adult patients with wide ranging neurologic diseases and found that ferritin is synthesized and secreted locally in the CNS, and that ferritin can also transudate from serum across an injured BBB (29). Using a murine model, Cohen et al. investigated the source of elevation in serum ferritin and demonstrated that it was largely due to macrophage secretion (33). Furthermore, Glanzer et al. reported that murine microglia in vitro secrete ferritin upon activation (34). Secretion from macrophages/microglia may occur in response to several stimuli following TBI. Ferritin is a key iron storage protein in the brain and is involved in iron homeostasis. Following TBI, an increase in free heme occurs due to release of hemoglobin and heme-proteins from hemorrhage and cellular damage. Heme degradation leads to increased free iron, which if excessive, can lead to secondary neurotoxicity via lipid peroxidation and free radical formation (35). Following TBI with ICH, brain ferritin levels increase substantially and the timing of increase parallels an increase in free iron suggesting that ferritin synthesis in the brain is strongly mediated by iron. Additionally, cytokines such as tumor necrosis factor- α and IL-1 β are known to stimulate ferritin secretion and have also been shown to be elevated in the CNS following TBI (36).

In our patients with severe TBI, ferritin was elevated at both time points studied when compared to control patients and to previously published control values (37). It could be that both local generation by activated macrophages/microglia and transudation of serum ferritin across an injured BBB contributes to elevated CSF levels of ferritin in our patients. When multivariate analysis was done, we found that high CSF ferritin levels were independently associated with young age and low initial GCS (3 or 4), suggesting that macrophage/microglia activation is more robust in younger children and in those with more severe injury. While co-linearity with young age exists, high CSF ferritin was also associated with AHT. This finding may be related to the prominence of subdural hematomas in this patient population (38), resulting in the presence of free heme, particularly in cases of repetitive injury with acute-on-chronic subdural hematomas. Following TBI, macrophages/microglia have been shown to develop a primed phenotype which would lead to the ability to respond more robustly to a second challenge (39). Further study will be needed to determine if inflammation is more robust in younger children with AHT and if this is related to priming of macrophages/microglia from previous, repetitive TBI. Although the purpose of this study was not to identify biomarkers predictive of outcome after TBI, high CSF ferritin was observed in the subgroup with unfavorable outcome. Thus, high CSF ferritin was identified not only in patients with poor outcome, but also in subgroups of patients known to be associated with poor outcome—young age, low GCS, and AHT. These data do not infer that therapeutic strategies targeting macrophage/microglial activation would be beneficial; however, they do offer a means for monitoring the mechanistic-based effectiveness of these therapies to directly address this question clinically.

Like ferritin, sCD163 has been studied extensively in the serum, but fewer studies have evaluated its role in the CNS. CD163 is a scavenger receptor expressed by monocytes and macrophages that serves predominantly as an endocytic receptor for hemoglobin-haptoglobin complexes functioning to clear free hemoglobin both in the serum and in the CNS. Under normal conditions, CNS expression of CD163 is restricted to perivascular

macrophages. However, parenchymal microglia have been shown to express CD163 during various diseases including viral encephalitis and multiple sclerosis (40). While membrane bound CD163 is considered a marker of anti-inflammatory macrophages, soluble CD163 is felt to be a marker of macrophage activation as several inflammatory stimuli have been shown to induce rapid shedding of CD163 (13). Furthermore, in diseases including sepsis and liver failure, elevated serum CD163 has been associated with severity of disease as well as a marker of poor prognosis (13, 41). In our patients, CSF sCD163 was elevated at the second time point analyzed compared to control levels and there was a significant rise in levels between the two time points assayed. However, we did not find any association between peak levels and outcome. This may have been due to timing of sample collection. In a study by Su et al. looking at the diagnostic value of serum biomarkers in sepsis, it was found that the dynamic change in sCD163 levels over 14 days as opposed to a single level was more reliable in predicting outcome (42).

While soluble IL-2R α levels were not elevated in our TBI patients compared to controls, sIL-2R α levels were highly correlated with ferritin and sCD163 levels. This suggests that macrophage/microglia activation may be coupled with T-cell activation in the immune response that follows TBI. Like CSF ferritin, high CSF sIL-2R α was associated with age 4 y by univariate analysis, suggesting that certain components of the immune response may be more robust in younger children; although this association was no longer apparent when controlling for other factors using multivariate analysis. In contrast to our findings, Lenzlinger et al. previously examined sIL-2R α levels following TBI in adults and found that the majority of patients had a mild elevation in sIL-2R α compared with controls. More in depth analysis examining interactions between sIL-2R α and other biomarkers of immune activation after TBI, and examining more delayed time points, may be more revealing.

Of significant interest in the present study is the association of young age with increased markers of both macrophage/microglia and T-cell activation following TBI. Importantly for pediatrics, the developmental stage of the brain may have significant impact on outcome following TBI. While children have better reported outcomes compared to adults following TBI, younger children have increased vulnerability to the effects of TBI (43, 44). In particular, children < 4 years-of-age have been reported to have worse outcomes compared with older children (45). It remains to be determined if this is due to differences in types of injury or due to differences in pathophysiology including secondary injury response pathways. Inflammation is a well-recognized mechanism of secondary injury following pediatric TBI and our current findings suggest that the inflammatory response following TBI may differ depending on the patient's age. This is consistent with our previous study showing that CSF levels of caspase-1, an enzyme that converts pro-IL-1 β to active IL-1 β , was also associated with younger age after pediatric TBI (19). Increasing attention is being paid to experimental models that investigate the effect of TBI specifically in the developing brain and may help elucidate mechanisms that may contribute to differences in neurological outcome in younger children (46–51). Our findings suggest that the response to therapies targeting neuroinflammation may be age-dependent.

Our study has limitations. Though our controls were selected from patients admitted for evaluation of febrile illness who ultimately had negative CSF cultures, it is possible that

these patients had viral encephalitis/meningitis or non-infectious inflammatory conditions that would impact biomarkers measured in CSF. Also, approximately half of our patients were enrolled in a concurrent Phase II trial of therapeutic hypothermia. Though no associations with CSF levels of ferritin, sCD163, or sIL-2Ra were seen between hypothermia and normothermia subgroups, it is possible that enrollment or lack of enrollment in a research study protocol may have confounded our results.

Conclusion

Markers of macrophage/microglial activation, ferritin and sCD163, are increased in CSF from pediatric patients after severe TBI. High CSF ferritin was associated with age < 4 y, low GCS, AHT, and unfavorable outcome, suggesting that neuroinflammation is more prominent in younger, more severely injured patients particularly after AHT, and that these patients are more likely to exhibit poor neurological outcome. Further study is warranted to determine whether macrophage/microglial activation following TBI in children represents a potential therapeutic target.

Acknowledgments

Support: NIH grants R01 NS38620 (RC), R01 GM098474, U01 NS081041 (MB), T32 HD40686 (DS), and R01 GM108618 (JAC)

References

1. Coronado VG, et al. Surveillance for traumatic brain injury-related deaths--United States, 1997–2007. *MMWR Surveill Summ.* 2011; 60(5):1–32. [PubMed: 21544045]
2. Das M, Mohapatra S, Mohapatra SS. New perspectives on central and peripheral immune responses to acute traumatic brain injury. *J Neuroinflammation.* 2012; 9:236. [PubMed: 23061919]
3. Beschoner R, et al. CD14 expression by activated parenchymal microglia/macrophages and infiltrating monocytes following human traumatic brain injury. *Acta Neuropathol.* 2002; 103(6): 541–9. [PubMed: 12012085]
4. Engel S, et al. Dynamics of microglial activation after human traumatic brain injury are revealed by delayed expression of macrophage-related proteins MRP8 and MRP14. *Acta Neuropathol.* 2000; 100(3):313–22. [PubMed: 10965802]
5. Scherbel U, et al. Differential acute and chronic responses of tumor necrosis factor-deficient mice to experimental brain injury. *Proc Natl Acad Sci U S A.* 1999; 96(15):8721–6. [PubMed: 10411942]
6. Sinz EH, et al. Inducible nitric oxide synthase is an endogenous neuroprotectant after traumatic brain injury in rats and mice. *J Clin Invest.* 1999; 104(5):647–56. [PubMed: 10487779]
7. Clark RS, et al. Neutrophil accumulation after traumatic brain injury in rats: comparison of weight drop and controlled cortical impact models. *J Neurotrauma.* 1994; 11 (5):499–506. [PubMed: 7861443]
8. Adelson PD, et al. Blood brain barrier permeability and acute inflammation in two models of traumatic brain injury in the immature rat: a preliminary report. *Acta Neurochir Suppl.* 1998; 71:104–6. [PubMed: 9779157]
9. Kumar A, et al. Traumatic brain injury in aged animals increases lesion size and chronically alters microglial/macrophage classical and alternative activation states. *Neurobiol Aging.* 2013; 34(5): 1397–411. [PubMed: 23273602]
10. Diamond ML, et al. IL-1 β associations with posttraumatic epilepsy development: A genetics and biomarker cohort study. *Epilepsia.* 2014; 55(7):1109–19. [PubMed: 24754437]
11. Lenzlinger PM, et al. The duality of the inflammatory response to traumatic brain injury. *Mol Neurobiol.* 2001; 24(1–3):169–81. [PubMed: 11831551]

12. Chandrakasan S, Filipovich AH. Hemophagocytic lymphohistiocytosis: advances in pathophysiology, diagnosis, and treatment. *J Pediatr*. 2013; 163(5):1253–9. [PubMed: 23953723]
13. Moller HJ. Soluble CD163. *Scand J Clin Lab Invest*. 2012; 72(1):1–13. [PubMed: 22060747]
14. Fitzgerald NE, McClain KL. Imaging characteristics of hemophagocytic lymphohistiocytosis. *Pediatr Radiol*. 2003; 33(6):392–401. [PubMed: 12768255]
15. Rooms L, Fitzgerald N, McClain KL. Hemophagocytic lymphohistiocytosis masquerading as child abuse: presentation of three cases and review of central nervous system findings in hemophagocytic lymphohistiocytosis. *Pediatrics*. 2003; 111(5 Pt 1):e636–40. [PubMed: 12728123]
16. Bell MJ, et al. Comparison of the interleukin-6 and interleukin-10 response in children after severe traumatic brain injury or septic shock. *Acta Neurochir Suppl*. 1997; 70:96–7. [PubMed: 9416290]
17. Bell MJ, et al. Interleukin-6 and interleukin-10 in cerebrospinal fluid after severe traumatic brain injury in children. *J Neurotrauma*. 1997; 14(7):451–7. [PubMed: 9257663]
18. Buttram SD, et al. Multiplex assessment of cytokine and chemokine levels in cerebrospinal fluid following severe pediatric traumatic brain injury: effects of moderate hypothermia. *J Neurotrauma*. 2007; 24(11):1707–17. [PubMed: 18001201]
19. Satchell MA, et al. Cytochrome c, a biomarker of apoptosis, is increased in cerebrospinal fluid from infants with inflicted brain injury from child abuse. *J Cereb Blood Flow Metab*. 2005; 25(7): 919–27. [PubMed: 15744250]
20. Shore PM, et al. Continuous versus intermittent cerebrospinal fluid drainage after severe traumatic brain injury in children: effect on biochemical markers. *J Neurotrauma*. 2004; 21(9):1113–22. [PubMed: 15453982]
21. Whalen MJ, et al. Soluble adhesion molecules in CSF are increased in children with severe head injury. *J Neurotrauma*. 1998; 15(10):777–87. [PubMed: 9814634]
22. Kochanek PM, et al. Guidelines for the acute medical management of severe traumatic brain injury in infants, children, and adolescents--second edition. *Pediatr Crit Care Med*. 2012; 13(Suppl 1):S1–82. [PubMed: 22217782]
23. Adelson PD, et al. Phase II clinical trial of moderate hypothermia after severe traumatic brain injury in children. *Neurosurgery*. 2005; 56(4):740–54. discussion 740–54. [PubMed: 15792513]
24. Kaneko Y, et al. Ferritin immunohistochemistry as a marker for microglia. *Acta Neuropathol*. 1989; 79(2):129–36. [PubMed: 2596262]
25. Todorich B, Zhang X, Connor JR. H-ferritin is the major source of iron for oligodendrocytes. *Glia*. 2011; 59(6):927–35. [PubMed: 21446040]
26. Holfelder K, et al. De novo expression of the hemoglobin scavenger receptor CD163 by activated microglia is not associated with hemorrhages in human brain lesions. *Histol Histopathol*. 2011; 26(8):1007–17. [PubMed: 21692033]
27. Liu HD, et al. Increased expression of ferritin in cerebral cortex after human traumatic brain injury. *Neurol Sci*. 2013; 34(7):1173–80. [PubMed: 23079850]
28. Zhang Z, et al. Lesional accumulation of CD163+ macrophages/microglia in rat traumatic brain injury. *Brain Res*. 2012; 1461:102–10. [PubMed: 22583855]
29. Keir G, Tasdemir N, Thompson EJ. Cerebrospinal fluid ferritin in brain necrosis: evidence for local synthesis. *Clin Chim Acta*. 1993; 216(1–2):153–66. [PubMed: 8222266]
30. Petzold A, et al. Markers for different glial cell responses in multiple sclerosis: clinical and pathological correlations. *Brain*. 2002; 125(Pt 7):1462–73. [PubMed: 12076997]
31. Connor JR, et al. Isoforms of ferritin have a specific cellular distribution in the brain. *J Neurosci Res*. 1994; 37(4):461–5. [PubMed: 8021970]
32. Hohnholt MC, Dringen R. Uptake and metabolism of iron and iron oxide nanoparticles in brain astrocytes. *Biochem Soc Trans*. 2013; 41(6):1588–92. [PubMed: 24256259]
33. Cohen LA, et al. Serum ferritin is derived primarily from macrophages through a nonclassical secretory pathway. *Blood*. 2010; 116(9):1574–84. [PubMed: 20472835]
34. Glanzer JG, et al. Genomic and proteomic microglial profiling: pathways for neuroprotective inflammatory responses following nerve fragment clearance and activation. *J Neurochem*. 2007; 102(3):627–45. [PubMed: 17442053]

35. Chang EF, et al. Heme regulation in traumatic brain injury: relevance to the adult and developing brain. *J Cereb Blood Flow Metab.* 2005; 25(11):1401–17. [PubMed: 15917748]
36. Meyron-Holtz EG, Moshe-Belizowski S, Cohen LA. A possible role for secreted ferritin in tissue iron distribution. *J Neural Transm.* 2011; 118(3):337–47. [PubMed: 21298454]
37. Kim YO, et al. Diagnostic capability of CSF ferritin in children with meningitis. *Pediatr Neurol.* 2003; 28(4):271–6. [PubMed: 12849879]
38. Shein SL, et al. Risk factors for mortality in children with abusive head trauma. *J Pediatr.* 2012; 161(4):716–722 e1. [PubMed: 22578583]
39. Hsieh CL, et al. Traumatic brain injury induces macrophage subsets in the brain. *Eur J Immunol.* 2013; 43(8):2010–22. [PubMed: 23630120]
40. Zhang Z, et al. Parenchymal accumulation of CD163+ macrophages/microglia in multiple sclerosis brains. *J Neuroimmunol.* 2011; 237(1–2):73–9. [PubMed: 21737148]
41. Feng L, et al. Clinical significance of soluble hemoglobin scavenger receptor CD163 (sCD163) in sepsis, a prospective study. *PLoS One.* 2012; 7(7):e38400. [PubMed: 22911680]
42. Su L, et al. Diagnostic value of dynamics serum sCD163, sTREM-1, PCT, and CRP in differentiating sepsis, severity assessment, and prognostic prediction. *Mediators Inflamm.* 2013; 2013:969875. [PubMed: 23935252]
43. Luerssen TG, Klauber MR, Marshall LF. Outcome from head injury related to patient's age. A longitudinal prospective study of adult and pediatric head injury. *J Neurosurg.* 1988; 68(3):409–16. [PubMed: 3343613]
44. Taylor HG, et al. Traumatic brain injury in young children: postacute effects on cognitive and school readiness skills. *J Int Neuropsychol Soc.* 2008; 14(5):734–45. [PubMed: 18764969]
45. Potts MB, et al. Traumatic injury to the immature brain: inflammation, oxidative injury, and iron-mediated damage as potential therapeutic targets. *NeuroRx.* 2006; 3(2):143–53. [PubMed: 16554253]
46. Adelson PD, et al. Morris water maze function and histologic characterization of two age-at-injury experimental models of controlled cortical impact in the immature rat. *Childs Nerv Syst.* 2013; 29(1):43–53. [PubMed: 23089934]
47. Kamper JE, et al. Juvenile traumatic brain injury evolves into a chronic brain disorder: behavioral and histological changes over 6months. *Exp Neurol.* 2013; 250:8–19. [PubMed: 24076005]
48. Robertson CL, et al. Cerebral glucose metabolism in an immature rat model of pediatric traumatic brain injury. *J Neurotrauma.* 2013; 30(24):2066–72. [PubMed: 24032394]
49. Cernak I, et al. Pathophysiological response to experimental diffuse brain trauma differs as a function of developmental age. *Dev Neurosci.* 2010; 32(5–6):442–53. [PubMed: 20948187]
50. Prins ML, et al. Repeat traumatic brain injury in the juvenile rat is associated with increased axonal injury and cognitive impairments. *Dev Neurosci.* 2010; 32(5–6):510–8. [PubMed: 20829578]
51. Ji J, et al. Lipidomics identifies cardiolipin oxidation as a mitochondrial target for redox therapy of brain injury. *Nat Neurosci.* 2012; 15(10):1407–13. [PubMed: 22922784]

Figure 1.

Box plots showing CSF levels of sCD163 (A), sIL-2Rα (B), and ferritin (C) from control patients and TBI patients at time 1 (17 ± 10 h) and time 2 (17 ± 15 h), as well as the average and peak values for both time points. Data are presented as median (line), 25–75th (box), 10–90th (whiskers), and 5–95th (dots) percentiles; * $P < 0.05$. Lower limits of detection defined by the manufacturer for sCD163, sIL-2Rα, and ferritin were 0.18 ng/ml, 10.0 pg/ml, and 7.5 ng/ml, respectively.

Figure 2.

Comparisons of individual biomarkers within concurrent CSF samples at time 1 (17 ± 10 h) and time 2 (17 ± 15 h). A. sCD163 vs. sIL-2Rα. B. Ferritin vs. sIL-2Rα. C. Ferritin vs. sCD163.

Table 1

Demographic data

Control patients	sCD163/sIL-2R α cohort	Ferritin cohort
<i>n</i>	17	9
Age, mean [range]	3.4 [1 mo-11 y] *	*
<hr/>		
Traumatic brain injury patients	sCD16/sIL-2R α cohort	Ferritin cohort
<i>n</i>	66	59
Age, mean [range]	6.0 [1 mo-16 y]	6.2 [1 mo-16 y]
Male, <i>n</i> (%)	37 (56.1)	36 (61.0)
Initial Glasgow Coma Scale score, median [IQ]	7 [4.25–8.75]	7 [5–8]
Mechanism of injury, <i>n</i>		
Motor vehicle collision	21	19
Abusive head injury	17	13
Pedestrian vs. motor vehicle	14	14
Fall	13	12
Other	1	1
Intracranial hemorrhage on head CT scan, <i>n</i> (%)	46 (75.4) *	41 (74.6) *
Randomized to hypothermia, <i>n</i> (%)	15 (22.7)	14 (23.7)
6 mo Glasgow Outcome Scale score, median [IQ]	4 [3–5] *	5 [3–5] *
5 (good)	30	28
4 (moderate disability)	10	9
3 (severe disability)	15	13
2 (vegetative)	0	0
1 (death)	8	6

* missing data points

Table 2

CSF sCD163 levels within demographic subgroups.

Traumatic brain injury subgroup	sCD163 ng/ml	Univariate <i>P</i> -value
	Median [25–75 th percentile]	
Age 4 vs. > 4 y	109.7 [47.9–136.7] vs. 80.0 [17.2–154.4]	0.441
Male vs. Female	80.0 [23.8–133.7] vs. 113.4 [46.9–142.8]	0.251
iGCS 4 vs. > 4 y	111.4 [29.5–141.8] vs. 95.4 [31.0–139.9]	0.548
AHT vs. Accidental	102.9 [34.9–131.1] vs. 103.1 [28.7–141.3]	0.872
ICH vs. No ICH	104.3 [33.5–139.4] vs. 52.7 [17.8–142.4]	0.561
GOS 1–3 vs. 4,5	102.9 [38.5–127.0] vs. 103.1 [28.3–141.3]	0.989
Normothermia vs. Hypothermia	99.2 [31.8–134.5] vs. 116.0 [33.0–143.2]	0.712

Abbreviations: AHT, abusive head trauma; CSF, cerebrospinal fluid; GOS, Glasgow Outcome Scale score; ICH, intracranial hemorrhage; iGCS, initial Glasgow Coma Scale score

Table 3CSF sIL-2R α levels within demographic subgroups.

Traumatic brain injury subgroup	sIL-2R α (pg/ml)	Univariate <i>P</i> -value
	Median [25–75 th percentile]	
Age 4 vs. > 4 y	116.8 [44.7–178.3] vs. 43.8 [18.7–97.0]	< 0.001
Male vs. Female	43.9 [21.0–132.1] vs. 67.6 [43.8–130.7]	0.174
iGCS 4 vs. > 4 y	81.9 [34.3–139.4] vs. 50.3 [22.9–119.1]	0.348
AHT vs. Accidental	70.8 [40.2–146.0] vs. 47.9 [21.8–129.1]	0.247
ICH vs. No ICH	65.0 [25.6–129.5] vs. 43.8 [20.8–130.0]	0.463
GOS 1–3 vs. 4,5	70.8 [37.9–150.9] vs. 46.0 [22.6–116.3]	0.247
Normothermia vs. Hypothermia	61.0 [36.9–119.1] vs. 39.5 [22.2–154.9]	0.845

Abbreviations: AHT, abusive head trauma; CSF, cerebrospinal fluid; GOS, Glasgow Outcome Scale score; ICH, intracranial hemorrhage; iGCS, initial Glasgow Coma Scale score

Table 4

CSF ferritin levels within demographic subgroups.

Traumatic brain injury subgroup	Ferritin (ng/ml)	Univariate <i>P</i> -value
	Median [25–75 th percentile]	
Age 4 vs. > 4 y	36.5 [14.7–56.0] vs. 9.1 [7.5–21.0]	0.001
Male vs. Female	12.7 [7.5–28.5] vs. 20.0 [9.4–63.4]	0.112
iGCS 4 vs. > 4 y	25.2 [17.8–49.2] vs. 10.5 [7.5–38.2]	0.024
AHT vs. Accidental	27.9 [7.5–68.5] vs. 13.6 [7.5–33.4]	0.285
ICH vs. No ICH	15.2 [7.5–39.1] vs. 12.8 [7.5–67.3]	0.971
GOS 1–3 vs. 4,5	27.9 [12.6–68.0] vs. 10.1 [7.5–27.4]	0.014
Normothermia vs. Hypothermia	19.2 [7.5–42.4] vs. 12.7 [7.5–17.2]	0.387

Abbreviations: AHT, abusive head trauma; CSF, cerebrospinal fluid; GOS, Glasgow Outcome Scale score; ICH, intracranial hemorrhage; iGCS, initial Glasgow Coma Scale score

Table 5

Multivariate logistic regression analysis comparing high CSF ferritin levels, defined as a level > median value for all TBI patients (8.32 ng/ml), with dichotomized clinical variables.

TBI* Patients (n=66)	Low Ferritin <i>n</i> (%)	High Ferritin <i>n</i> (%)	OR [5–95% CI]	<i>P</i>
Age 4 y (n=25)	5 (20)	20 (80)	8.106 [1.147–57.295]	0.036
Male (n=36)	15 (42)	21 (58)	0.174 [0.0282–1.072]	0.059
Initial GCS 3, 4 (n=13)	0 (0)	13 (100)	†	-
AHT (n=13)	4 (31)	9 (62)	34.097 [1.646–706.44]	0.022
Hemorrhage on head CT (n=41)	11 (27)	30 (73)	1.307 [0.182–9.372]	0.790
GOS 1–3 (19)	3 (16)	16 (84)	19.777 [1.061–368.623]	0.046

* Abbreviations: Abusive head trauma, AHT; Computerized tomography, CT; Glasgow coma scale score, GCS; Glasgow outcome scale score, GOS; Traumatic brain injury, TBI

† Unable to calculate given absence of patients with initial GCS 3, 4 and low ferritin.