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Plasma Levels, Temporal Trends and Clinical Associations between Biomarkers of Inflammation and Vascular Homeostasis after Pediatric Traumatic Brain Injury

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Abstract

Expression of inflammatory (interleukin-6 [IL-6]) and vascular homeostatic (angiopoietin-2 [AP-2], endothelin-1 [ET-1], endocan-2 [EC-2] biomarkers in pediatric traumatic brain injury

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(TBI) was examined in this prospective, observational cohort study of 28 children hospitalized with mild, moderate and severe TBI by clinical measures (age, sex, Glasgow Coma Scale score [GCS], Injury Severity Score [ISS], and cerebral autoregulation status). Biomarker patterns suggest an inverse relationship between GCS and AP-2, GCS and IL-6, ISS and ET-1, but a direct relationship between GCS and ET-1 and ISS and AP-2. Biomarker patterns suggest an inverse relationship between AP-2 and ET-1, AP-2 and EC-2, but a direct relationship between AP-2 and IL-6, IL-6 and EC-2, and IL-6 and ET-1. Plasma concentrations of inflammatory and vascular homeostatic biomarkers suggest a role for inflammation and disruption of vascular homeostasis during the first ten days across the severity spectrum of pediatric TBI. Although not statistically significant, without impact on cerebral autoregulation, biomarker patterns suggest a relationship between inflammation and alterations in vascular homeostasis. The large variation in biomarker levels within TBI severity and age groups, and by sex suggests other contributory factors to biomarker expression.

Keywords

angiopoietin-2; endocan-1; endothelin-1; interleukin-6; children; traumatic; brain injury; endothelium

Introduction

Traumatic brain injury (TBI) is a leading cause of death and disability and affects 47–280 of 100,000 children worldwide.(1) In the United States alone, an estimated 600,000 children under the age of 18 years are hospitalized, of whom 7600 die, and another 5000 are disabled each year.(2, 3) Poor outcomes after TBI are associated with initial injury, as well as secondary injury from inflammation and vascular dysregulation.(4) Secondary brain injury consists of processes that are initiated in response to hypoxia, ischemia, or excitotoxic injury to neurons and astroglial cells, and biomarkers in plasma and cerebrospinal fluid may allow us to study these complex cascades after pediatric TBI.(5–9) However, biomarker expression of vascular homeostatic or inflammatory processes is not well characterized in children with TBI.

Angiopoietin-2(AP-2) is a member of a family of vascular growth factors that plays a role in angiogenesis, and in controlling microvascular permeability, vasodilation, and vasoconstriction by signaling vascular smooth muscle cells.(10) Increase in AP-2 promotes inflammation, and changes in AP-2 occur during illnesses(11), (12) including after cerebral ischemia.(13, 14) Angiopoietin also modulates BBB breakdown and endothelial apoptosis, (15) and early phase of post-injury BBB breakdown is associated with a reduction in angiopoietin-1 and upregulation of AP-2.(15) However, patterns of expression after pediatric TBI have not been reported. Endothelin-1 (ET-1) is an endogenous vasoconstrictor peptide that plays a role in vascular homeostasis.(16–18) Endothelin-1 is implicated in developmental outcomes of very low birth weight newborns with hypoxic encephalopathy, (19) blast-induced TBI in rats,(20) delayed cerebral hypoperfusion after global ischemia, (21) and overexpression of ET-1 may contribute to dementia associated with ischemic stroke by exaggerating astrocyte-derived amyloid secretion, (22) but its role in pediatric TBI is

unknown. Endocan-2 (EC-2), also known as endothelial cell-specific molecule-1 may play a role during neoangiogenesis, and levels of endocan are increased in the presence of pro-angiogenic growth factors such as vascular endothelial growth factor or fibroblast growth factor. While endocan has been studied in cancer and pre-eclampsia,(23, 24) there are no reports in TBI.

Cerebral inflammatory stimuli such as hypoxia and ischemic-reperfusion may stimulate biosynthesis of interleukins such as interleukin-6 (IL-6) in endothelium, glia, and leukocytes. Interleukins increase vascular permeability, modify the blood-brain barrier, and lead to leukocyte accumulation. Interleukin 6 activity has been implicated in preclinical and adult models of TBI (25),(26),(27) and may be expressed in the cerebrospinal fluid (4, 7, 28, 29) as well as the serum.(7, 30, 31) The expression of IL-6 in the serum may decrease at the end of one week after TBI (31), and may be associated with both, favorable (31) and unfavorable outcomes after pediatric TBI. (7)

Given prior work in preclinical models of TBI (32) as well as the paucity of such information in pediatric TBI, we sought to understand the plasma levels, patterns and relationships between inflammatory and vascular homeostatic biomarker expression in pediatric TBI.

Methods

Study center and data sources

A prospective observational cohort study was performed between on a convenience sample at Harborview Medical Center, which is a level I pediatric trauma center. This study was conducted between September 2016 and September 2018. Only hospitalized children under 18 years with a history of trauma, with evidence of TBI on computerized tomography (CT) of the head were eligible for inclusion. Pregnant minors, children under child protective services, patients with ongoing cardiopulmonary resuscitation, or patients with brain death were excluded per suggestion of the Institutional Review Board and anticipated challenges with obtaining blood samples. Research coordinators screened admission logs daily for eligible subjects. Informed consent was obtained from the patient's legally authorized representative, and assent was obtained from patients as appropriate, and as soon as possible from the time of hospital admission (Figure 1). Data from some patients in cohort presented in this study have been previously published.(33) The study was approved by the Institutional Review Board at the University of Washington.

Data collection

Biomarker assay—In tandem with venipuncture performed for clinical care, blood was collected for the study into 6 mL vacutainers (BD K_2EDTA, 10.8 mg/tube, City, State) and after inverting ten times, it was immediately placed on ice. Blood samples were then centrifuged at 2380 RPM for 11 minutes within two hours of the draw. Plasma was removed from the tube after centrifugal fractionation and aliquoted into 0.5 mL cryovials and frozen at −80 C in a freezer for long-term storage. EMD Millipore's MILLIPLEX MAP assays

were used to quantify Endocan-1,(34) Angiopoietin-2, Endothelin-1,(35) and IL-6.(36) Plasma biomarker levels are expressed in pg/ml.

Clinical data—Demographic and clinical data were abstracted from the hospital electronic medical record. Admission head computerized tomography (CT) imaging reports were reviewed to classify radiographic characteristics of TBI (i.e., subdural hematoma, epidural hematoma, subarachnoid hemorrhage, intraparenchymal hemorrhage, cerebral edema, cerebral herniation, skull fracture, subgaleal hematoma, pneumocephalus or intraventricular hemorrhage).

TBI versus Other Pediatric Conditions—We reviewed published studies in pediatric TBI, other pediatric disease states, in healthy children and adolescents. Using PubMed, a search was conducted using the following search words: angiopoietin-2, endothelin-1, endocan-2, and interleukin-6. The search was restricted between 1975 and 2018, and to studies including patients between 0 and 18 years of age.

Statistical Analysis

Based on admission Glasgow Coma Score (GCS), TBI severity was categorized as mild (GCS 13–15), moderate (GCS 9–12), and severe (GCS 3–8). Patients were grouped by age: (1) 0–4 years, (2) 5–9 years, (3) 10–14 years, and (4) 15–18 years. Lesions on head CT were grouped as isolated and mixed (more than one lesion). Further, these were divided into extra-axial hemorrhage (SDH/ EDH), and intra-axial hemorrhage (SAH, IPH, IVH). Details on cerebral autoregulation testing on this cohort have been previously described (33). Patients who had TCDs were defined with impaired (ARI < 0.4) and normal cerebral autoregulation (ARI >=0.4).

Since data were not normally distributed, Wilcoxon rank-sum was used to examine the relationship between the four biomarkers (AP-2, ET-1, EC-2, IL-6), as well as relationship of each biomarker and a) TBI severity, b) age groups, c) head CT lesions, and 4) sex. Individual concentrations and patterns of the relationships between the four biomarkers were examined in relation to GCS and ISS and tested using the nonparametric Spearman's (rho) rank correlation analysis. We examined biomarker concentrations by age group, and in comparison, to healthy controls, and specific disease types.

P-values < 0.05 were considered significant. Summary data and the relationship between biomarker levels and TBI characteristics are presented as n (%), median [IQR]. Multivariable linear regression was conducted to examine the impact of impaired autoregulation on levels of each of the four biomarkers, adjusting for TBI severity. Data are presented as coefficients with 95% confidence intervals. Analysis was conducted using Stata 13.1(College Station, TX).(37)

Results

Patient Characteristics

Table 1 summarizes demographics and illness severity of the 28 participants, who were 11[IQR: 5 – 15.5] years old, mostly male (64.3%), with ISS 25[IQR: 17 – 27 10], with

median admission GCS 11[IQR: 6 – 15]. Upon hospital admission, 14 children had mild TBI, 3 children had moderate TBI, and 11 children had severe TBI. Overall, intensive care unit length of stay was 1.9[IQR: 1.1 – 5.6] days, hospital length of stay was 6.5[IQR: 1.7 – 11.5] days, discharge Glasgow Coma Score (GCS) was 15[IQR: 15 – 15]. Twenty (71.4%) patients were discharged home, 7(25%) patients were discharged to in-patient rehabilitation facility, and 1(3.6%) expired.

Participants underwent a total of 64 blood draws between hospital days 1 and 10 as follows: day 1 (n=7), day 2 (n=14), day 3 (n=13), day 4 (n=5), day 5 (n=10), day 6 (n=5), day 7 (n=3), day 8 (n= 4), day 9 (n= 2), and day 10 (n= 1). Seventeen children had repeat sampling between days 1 and 10.

Plasma Biomarker Levels

Overall, Initial and Temporal Trends—Table 2 shows that for the full cohort of children, median biomarker levels over the first 10 days were: AP-2 (2610.7; range 1282.36–10392.66pg/ml), ET-1 (92.97; range 25.86–502.5 pg/ml), EC-2 (2032.76; range 511.07–5625.63 pg/ml), and IL-6 (23.37; range 6.26–180.54 pg/ml).

Although there were no statistically significant differences in biomarker expression either between day 1-day 3 vs. day 4-day 10 or between day 1-day 7 vs. day 8-day 10, Figure 2 suggests that compared to mild and moderate TBI, patients with severe TBI have higher levels of AP-2, IL-6, and EC-2 through day 10 after admission.

Patterns in Biomarker Expression

Clinical Characteristics and Biomarker Expression—Biomarker levels were comparable across the TBI severity spectrum (Figure 3) across all four biomarkers. Table 2 shows large variation in median AP-2, median ET-1, median EC-2 and median IL-6 levels within TBI severity groups and no significant difference byTBI severity (Figure 3).

Median biomarker levels did not vary significantly by TBI severity, age group, or sex; (Figure 3, and Table 2) However, biomarker patterns suggest an inverse relationship between GCS and AP-2, GCS and IL-6, ISS and ET-1, but a direct relationship between GCS and ET-1 and ISS and AP-2. (Figure 4). However, none of these relationships were statistically significant.

Biomarker Levels and CT lesions—Five (18%) patients had isolated lesion on CT: subdural hematoma (n = 2), epidural hematoma (n = 1), intraparenchymal hemorrhage (n =1), and diffuse axonal injury (n = 1). Seventeen patients had extra-axial hemorrhage while nine patients had extra and intra-axial hemorrhage. Levels of AP-2, ET-1, EC-2, and IL-6 associated with isolated and mixed CT lesions are presented in Table 2. There was no difference in biomarker levels by head CT lesion type.

Relationship between Plasma Biomarkers—Biomarker patterns suggest an inverse relationship between AP-2 and ET-1, AP-2 and EC-2, but a direct relationship between AP-2 and IL-6, IL-6 and EC-2, and IL-6 and ET-1(Figure 5).

Association between Plasma Biomarkers and Cerebral autoregulation—As shown in Table 3, after adjusting for TBI severity, we found no statistically significant association between biomarker expression and cerebral autoregulation status.

Comparison of Biomarker Expression to Other Conditions: Figure 6 shows an overlap between AP-2, ET-1 and IL-6 biomarker levels and published data from non-TBI cohorts and age-matched controls. No reports of EC-2 levels were found.

Discussion

The purpose of this preliminary study was to explore the expression of inflammatory and vascular homeostatic plasma biomarkers in children hospitalized with TBI. While IL-6 has been studied to a limited extent, this is the first study to report on AP-2, ET-1, and EC-2, and to examine the levels, patterns, and the interplay between these biomarkers in pediatric TBI. Our findings are that: (1) Although not statistically significant, levels of inflammatory and vascular homeostatic biomarker expression suggests abnormalities in inflammation and vascular homeostasis during the first 10 days related to TBI severity, (2) Patterns of biomarker expression suggest an interplay between inflammation and vascular homeostasis, (3) The variation in biomarker levels within TBI severity, age, and by sex suggests other contributory factors to biomarker expression, and (4) There is no relationship between biomarker expression and cerebral autoregulation status.

Broadly, biomarkers relevant to TBI can be categorized as related to inflammatory(*interleukins, marinobufagenin*), traumatic neuronal/ axonal (*tau protein, neurofilaments, neuron-specific enolase[NSE], glial fibrillary acid protein[GFAP]),* myelin basic protein[MBP], ubiquitin carboxyl-terminal hydrolase isoenzyme L1[UCHL1*)*), spectrin breakdown products, neutrophil gelatinase-associated lipocalin (NGAL), blood-brain barrier integrity (*angiopoietin-1, angiopoietin-2,(15) cerebrospinal fluid/ plasma albumin ratio, tight junction proteins, NSE, S100B, GPAP*), and genetic biomarkers such as apolipoprotein-E [APO-E], and brain-derived neurotrophic factor [BDNF].(9)

We targeted the four biomarkers to examine inflammatory and vascular homeostatic pathways because of prior work showing alterations in these pathways and because these complex secondary TBI pathways may be related and or differentially expressed in pediatric TBI. Results from this study imply that EC-2 may not be expressed and be as representative of vascular homeostatic processes as perhaps AP-2, and ET-1 but these results need to be further confirmed.

Although not statistically significant, this study suggests that compared to mild TBI, there may be greater upregulation of inflammation and vascular homeostatic processes in severe TBI. A prior study on children with TBI by Chiaretti et.al. reported increases in plasma IL-6 levels with worsening TBI severity.(7) The pattern similarities between AP-2 and IL-6 suggests that both inflammation and blood-brain-barrier breakdown occurs with worsening TBI severity, and AP-2 may be considered as a surrogate marker of injury severity, similar to previous studies reporting higher levels of AP-2 in septic shock compared to sepsis,(38, 39) and as reported with IL-6.(4) Present results imply that AP-2 and IL-6 may also be surrogate

markers of pediatric TBI severity, but this will require confirmation in larger cohort of children with pediatric TBI. Despite the lack of significance, the present study is the first to report on the relationship between AP-2, ET-1, EC-2 by and across TBI severity.

Results of this study suggest no relationship between levels of AP-2 and IL-6 with TBI severity, age, and sex. However, age-related increases in NSE, S100B, and MBP have been described previously,(40) and we cannot exclude age-dependent increase in biomarker levels due to an age-dependent increase in blood-brain barrier permeability. Prior work by van Engelen et.al. examining S100B, NSE, and MBP in a study of 79 specimens of CSF showed no sex differences in TBI, (40) and there is no study examining the relationship with IL-6.(7) Present results suggest that interpretation of plasma biomarker data in pediatric TBI may be incomplete without knowledge of age, and sex of the population studied. Since clinical outcomes after pediatric TBI may be influenced by age and sex,(41) future studies should validate our findings in a larger population of children with TBI that includes sufficient numbers of males, females and age groups. We speculate that inflammatory and vascular homeostatic processes extend beyond the initial critical period after severe pediatric TBI (i.e. the first 72 hours), implying that these children may continue to remain at high risk for secondary brain insults from these processes beyond the first week after injury

Mechanistically, IL-6 is an inflammatory biomarker, and AP-2 and ET-1 are markers of blood-brain-barrier breakdown(15) and endothelial dysfunction,(12) respectively. Thus, the interplay between these biomarkers deserves some discussion. Whether the biomarkers follow direct or inverse relationships, and whether and how they interact to impact cerebral autoregulation is unknown. Future work is needed to confirm these biomarker relationships and effects.

Limitations

This preliminary study has some limitations. Although we examined biomarker expression across the TBI severity spectrum for initial assessment, we did not collect data from age-matched healthy controls. Due to the small sample size, our data are primarily descriptive, and although children in the moderate TBI group are very few, we felt it important to present biomarker levels for this group of patients separately. Despite these limitations, we provide new data on patterns of plasma biomarker expression reflecting inflammation and vascular homeostasis in children hospitalized with TBI.

Conclusions

Plasma concentrations of inflammatory and vascular homeostatic biomarkers suggest a role for inflammation and disruption of vascular homeostasis during the first 10 days across the severity spectrum of pediatric TBI. Although not statistically significant, and without impact on cerebral autoregulation, biomarker patterns suggest a relationship between inflammation and alterations in vascular homeostasis. The large variation in biomarker levels within TBI severity and age groups, and by sex suggests other contributory factors to biomarker expression.

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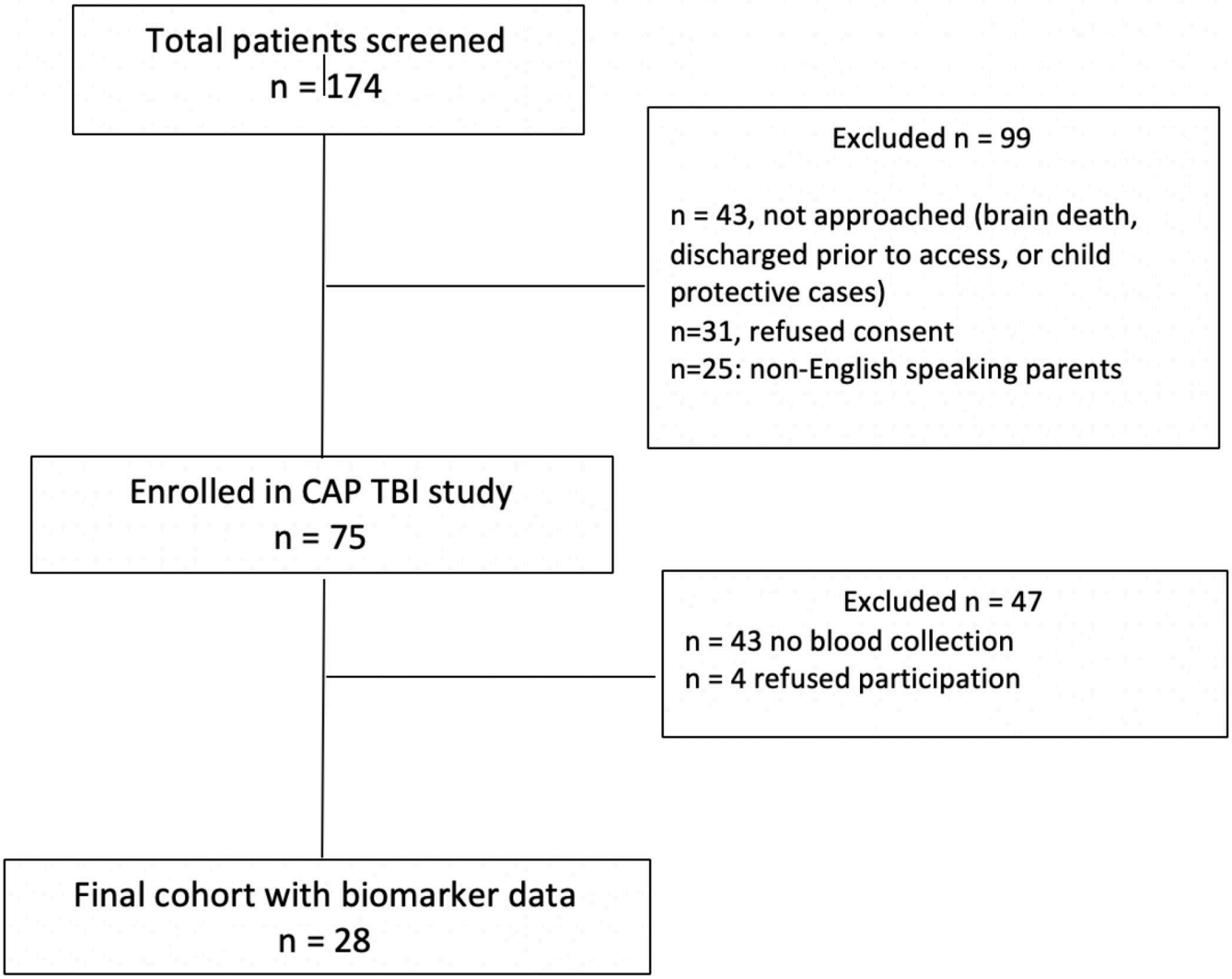


Figure 1.
Flow Diagram for Patient Selection for Biomarker Assays after Pediatric Traumatic Brain Injury

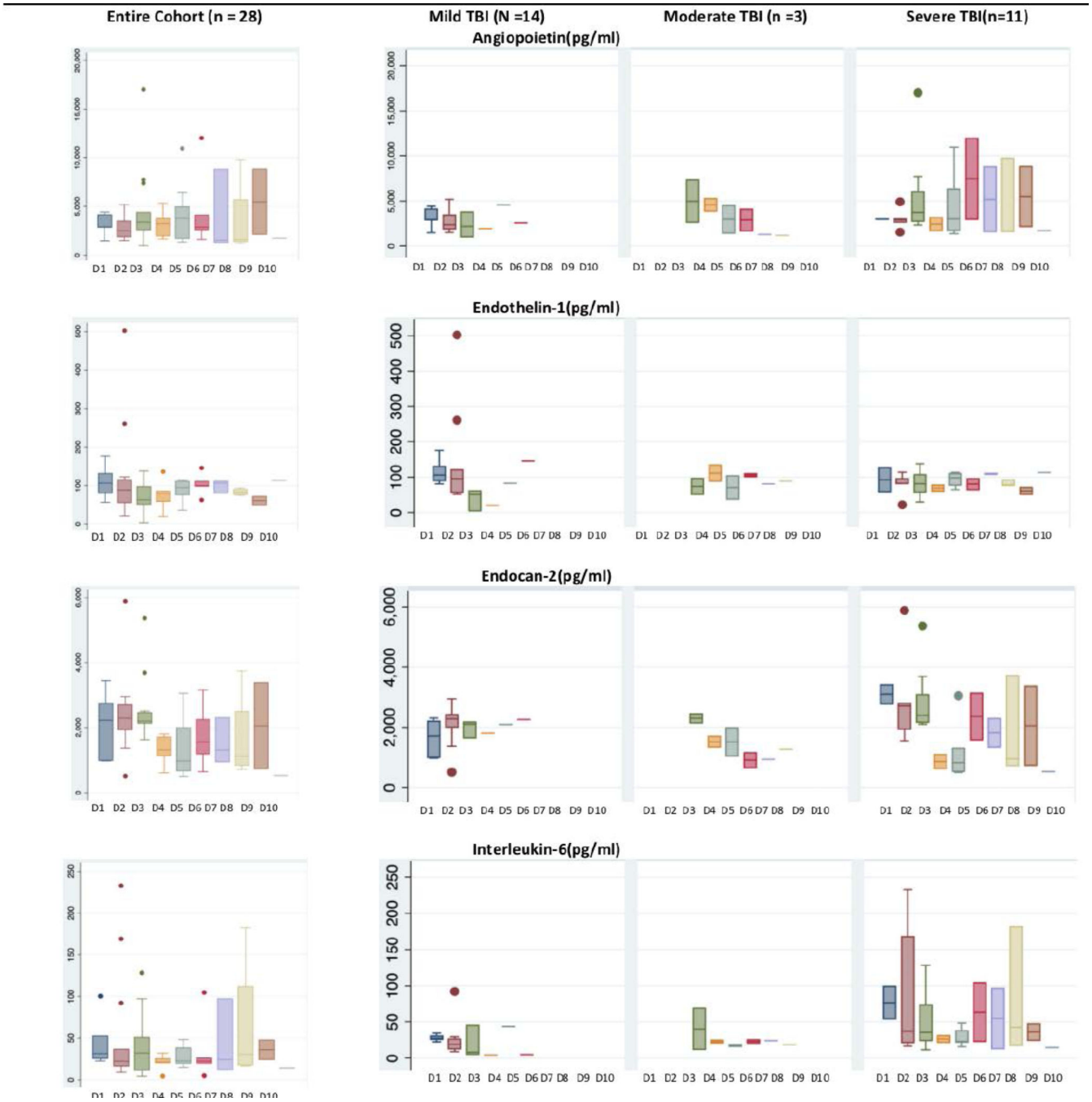


Figure 2.

Patterns of Plasma Biomarker Expression in 28 Children by Day of Admission by Severity of Traumatic Brain Injury and by Day of Admission Mild TBI: Admission GCS 13–15, Moderate TBI: Admission GCS: 9–12, Severe TBI: Admission GCS: 3–8 D1 = Admission day one, D10 = Admission day ten Data suggest that compared to mild and moderate TBI, patients with severe TBI have higher levels of AP-2, IL-6, and EC-2 through day 10 after admission

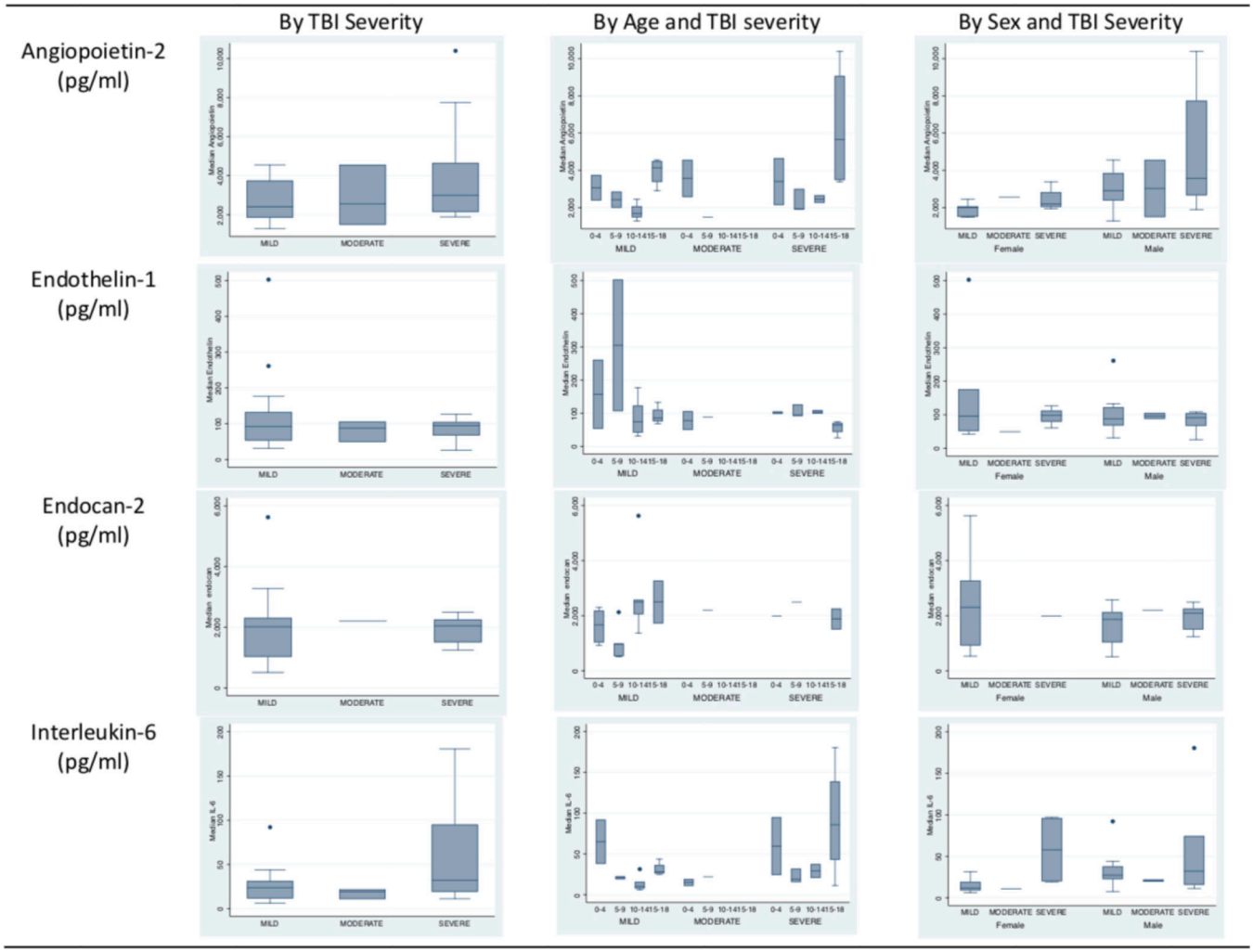


Figure 3.

Patterns of Plasma Biomarkers in 28 Children by and Traumatic Brain Injury (TBI) Severity, Age, and Sex Mild TBI: Admission Glasgow Coma Score (GCS): 13 −15, n = 11, Moderate TBI: Admission GCS: 9–12, n = 3, Severe TBI: Admission GCS: 3–8, n = 14 Age groups 0–4 years, 5–9 years, 10–14 years and 15–18 years Median biomarker levels did not vary significantly by TBI severity, age group, or sex

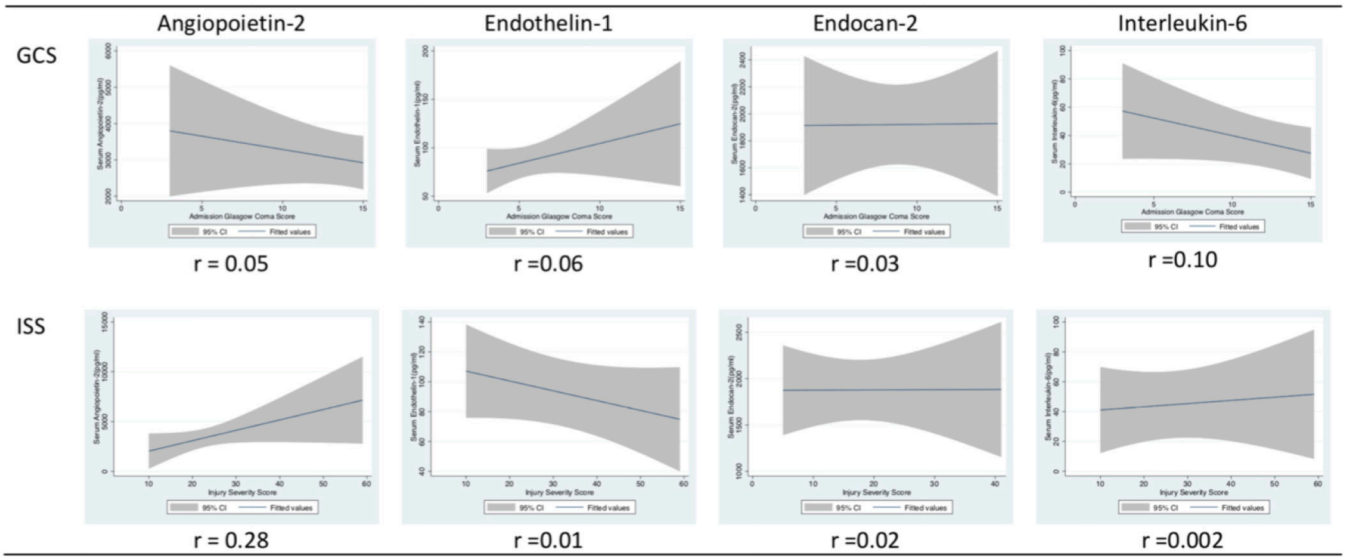


Figure 4.

Patterns of Plasma Biomarkers in 28 Children with Traumatic Brain Injury (TBI) by Glasgow Coma Score (GCS) and Injury Severity Score (ISS) Biomarker patterns suggest an inverse relationship between GCS and AP-2, GCS and IL-6, ISS and ET-1, but a direct relationship between GCS and ET-1 and ISS and AP-2.

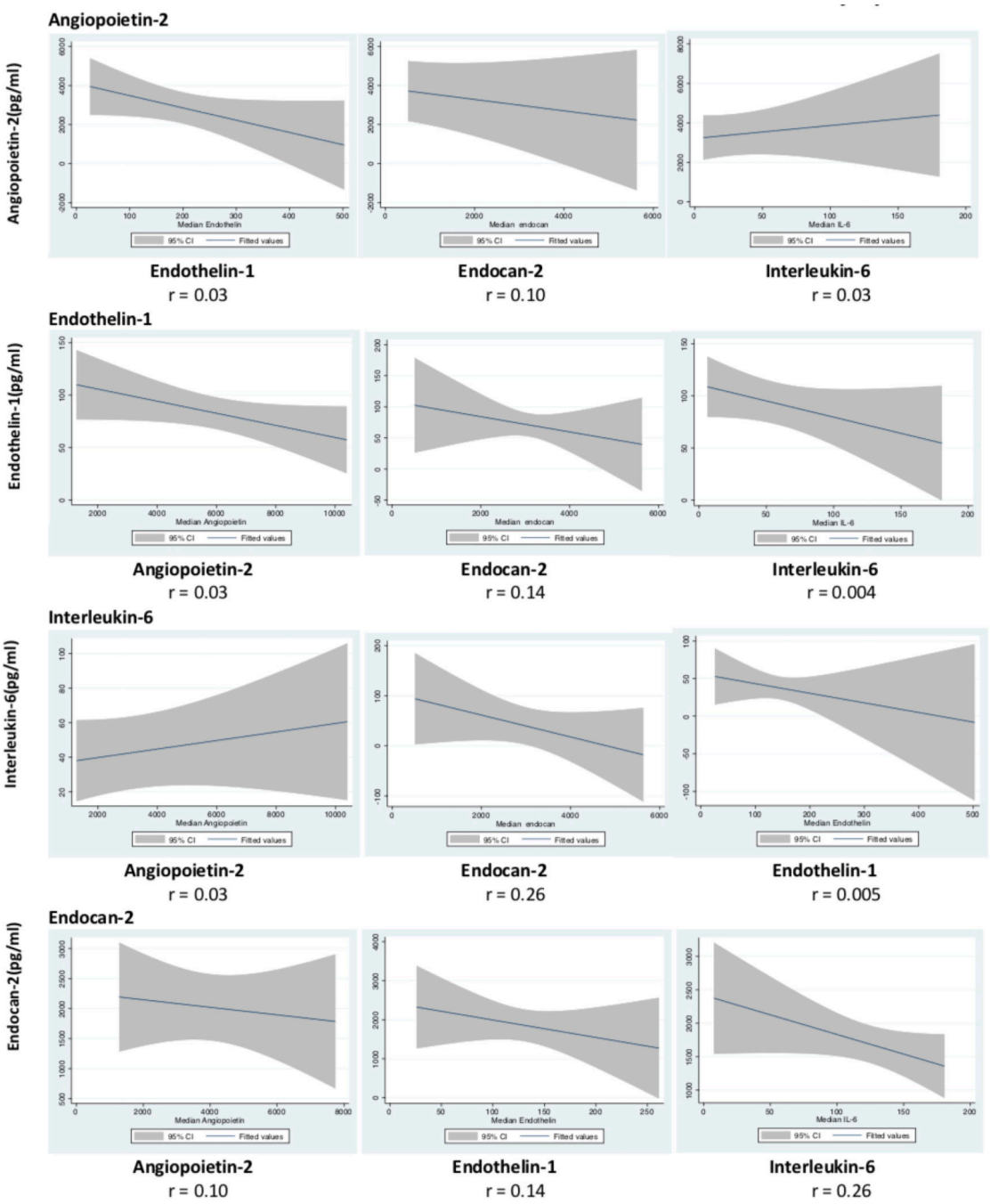
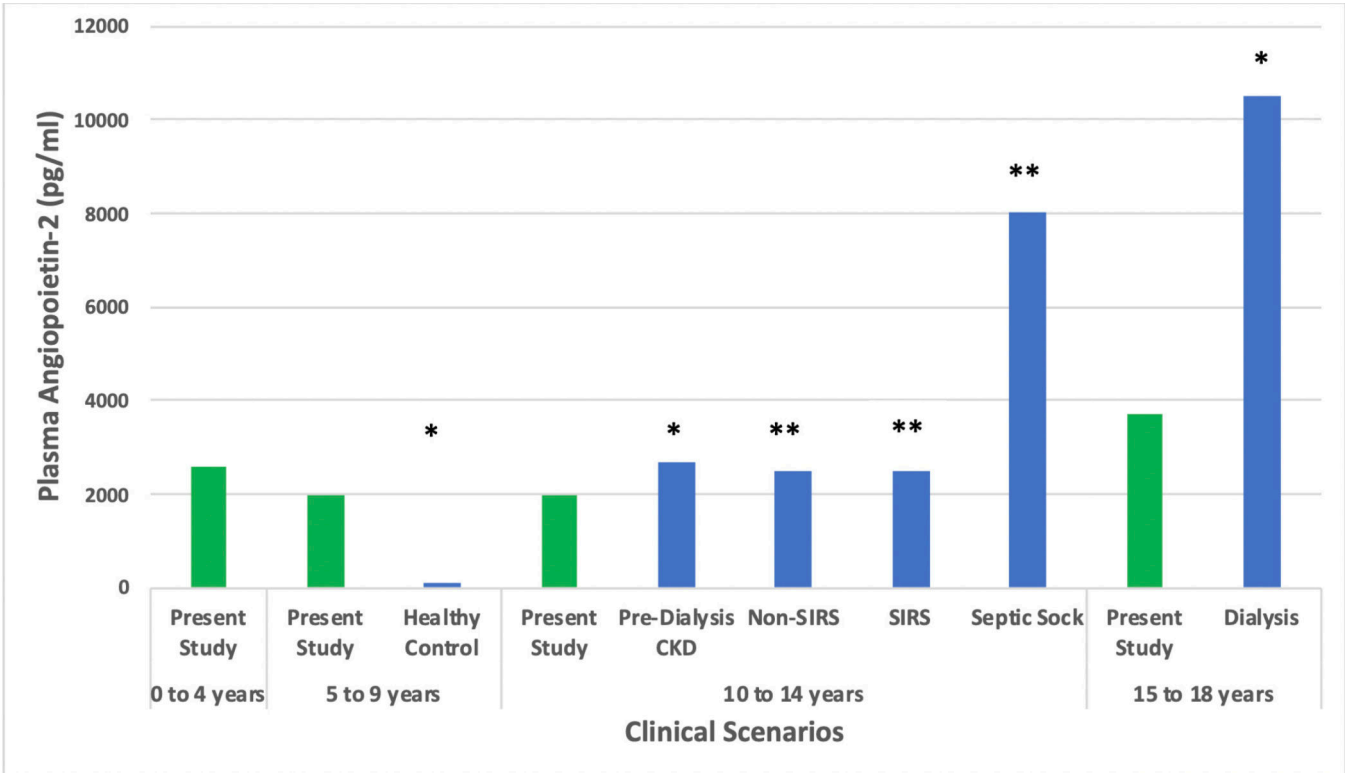
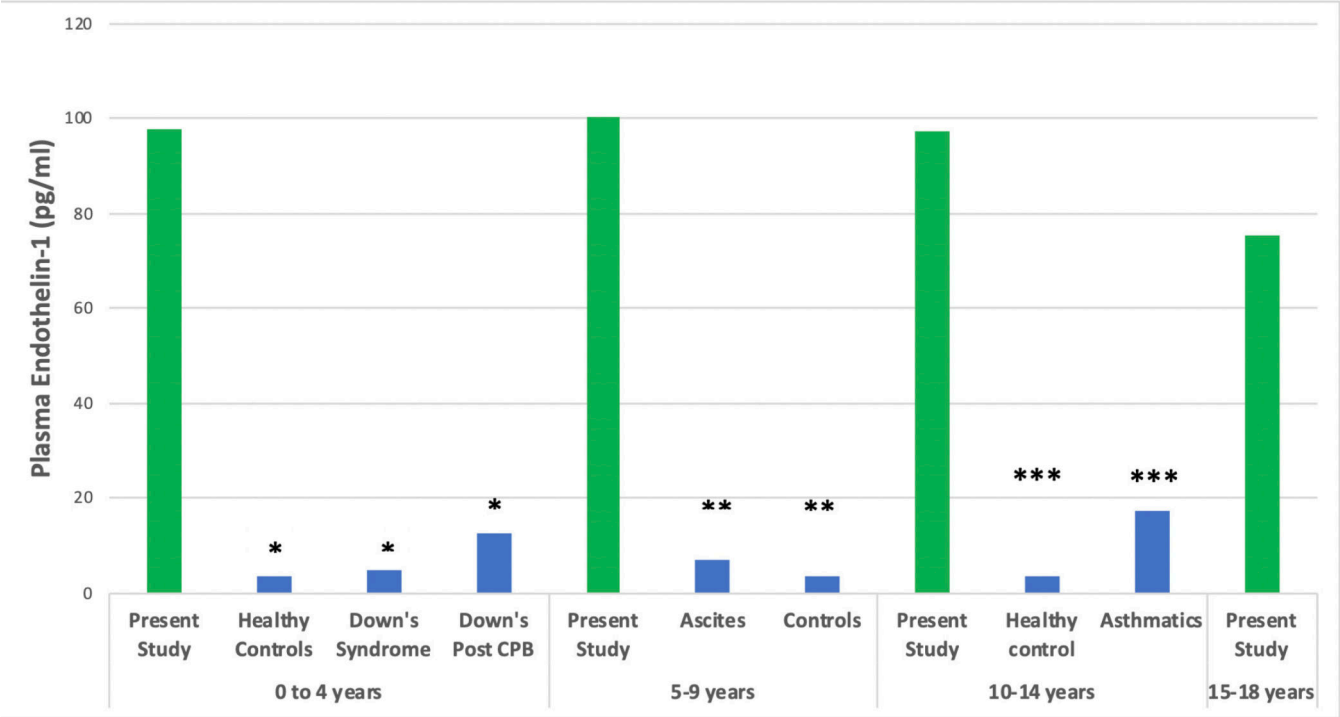


Figure 5.

The Interplay between Plasma Angiopoietin-2, Endothelin-1, Endocan-2 and Interleukin-6

Notes: Weighted by TBI Severity Biomarker patterns suggest an inverse relationship between AP −2 and ET-1, AP-2 and EC-2, but a direct relationship between AP-2 and IL-6, IL-6 and EC-2, and IL-6 and ET-1.





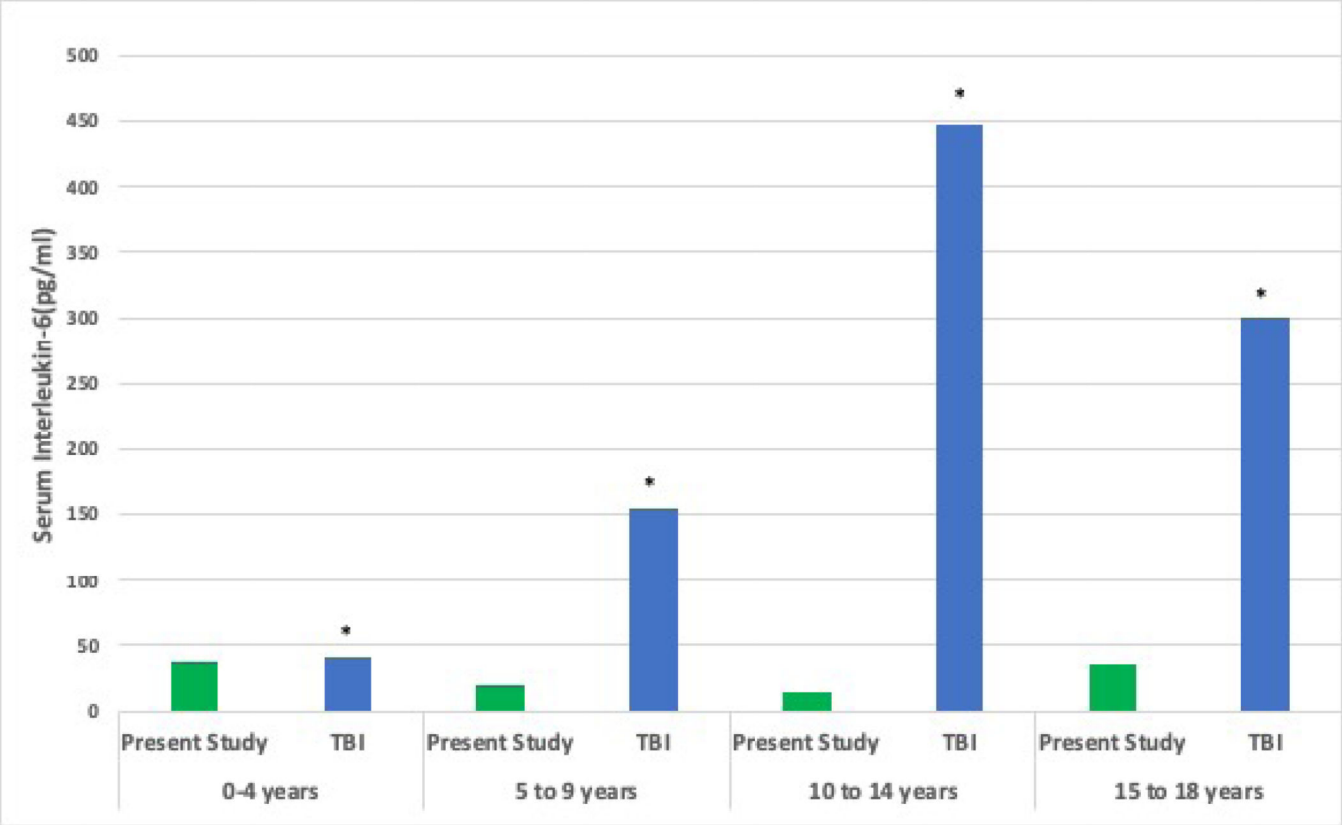


Figure 6.

Comparison of Median Plasma Biomarker Levels with Prior Work in Traumatic Brain Injury (TBI) and non-TBI cohorts a Plasma Angiopoietin-2 Comparison studies * 2013, PLoS One. 2013;8(2): e56273. ** 2007, Shock. Dec; 28(6): 650–654 Notes: CKD: chronic kidney disease, SIRS: systemic inflammatory response syndrome b Plasma Endothelin-1 Comparison studies * 2007, Paediatr Anaesth 17(11): 1071–1077. ** 2002, J Pediatr Gastroenterol Nutr 35(2): 149–153. *** 2002, Ann Allergy Asthma Immunol 88(4): 370–373. Notes: CPB: cardiopulmonary bypass c Plasma Interleukin-6 Comparison study: * 2005, Childs Nerv Syst 21(3): 185–193; discussion 194. TBI: traumatic brain injury In the present study, number of patients per age group were; 0–4 years: n=6, 5–9 years: n=6, 10–14 years: n=8, 15–19 years: n =8.

Table 1

Characteristics of Children (n = 28) with Mild* (n =14), Moderate** (n=3) and Severe *** (n =11) Traumatic Brain Injury (TBI)

Parameter	All Patients (n = 28)	Mild TBI (n = 14)	Moderate TBI (n =3)	Severe TBI (n =11)
Age in years median [IQR]	11[5, 15.5]	12[9, 15]	3[1, 7]	11[5, 16]
Age groups				
0–4 years	6(21.4%)	2(14.3%)	2(66.7%)	2(18.2%)
5–9 years	6(21.4%)	2(14.3%)	1(33.3%)	3(27.3%)
10–14 years	8(28.6%)	6(42.8%)	0	2(18.2%)
15–18 years	8(28.6%)	4(28.6%)	0	4(36.3%)
Male	18 (64.3%)	9(64.3%)	2(66.7%)	7(63.7%)
Injury Severity Score (ISS), median [IQR]	25[17, 27]	17.5[11, 26]	41[26, 41]	21.5[17, 27]
Admission Glasgow Coma Score (GCS), median [IQR]	13[6, 15]	15[14, 15]	9[9, 9]	4.5[3, 6]
Mechanism of Injury				
Motor Vehicle crash	10(35.7%)	2(14.3%)	1(33.3%)	7(63.6%)
Fall	10(35.7%)	7(70%)	0	3(30%)
Struck by or against	6(21.4%)	5(83.3%)	0	1(16.7%)
Other	2(7.1%)	0	2(100%)	0
Intracranial Lesion on CT head				
Isolated Skull fracture Skull fracture +	0 15(53.6%)	9(64.3%)	2(66.7%)	4(36.4%)
Isolated Subgaleal hematoma Subgaleal hematoma +	0 13(46.4%)	6(42.9%)	1(33.3%)	6(53.6%)
Isolated Subdural hematoma Subdural hematoma +	2(7.1%) 12(42.9%)	1(7.1%) 8(57.1%)	1(33.3%) 0	4(36.4%)
Isolated Subarachnoid hemorrhage Subarachnoid hemorrhage +	0 12(42.9%)	6(42.9%)	0	6(54.6%)
Isolated Epidural hematoma Epidural hematoma +	1(3.6%) 7(25%)	4(28.6%)	1(33.3%)	1(9.1%) 2(18.2%)
Isolated Pneumocephalus Pneumocephalus +	0 7(25%)	3(21.4%)	2(66.7%)	2(18.2%)
Isolated Intraparenchymal hemorrhage Intraparenchymal hemorrhage +	1(3.6%) 3(10.7%)	1(7.1%) 2(14.3%)	0	1(9.1%)
Isolated Cerebral edema Cerebral edema +	0 4(14.3%)	1(7.1%)	0	3(27.3%)
Isolated Cerebral herniation Cerebral herniation +	0 1(3.6%)	0	0	1(9.1%)
Isolated Diffuse axonal injury Diffuse axonal injury +	0 1(3.6%)	0	0	1(9.1%)
Isolated Intraventricular hemorrhage Intraventricular hemorrhage +	0 1(3.6%)	0	0	1(9.1%)
Intensive care unit length of stay, days, median [IQR]	1.9[1.1, 5.6]	1.6[1, 1.8]	4.1[1, 5.8]	4.6[2.1, 10.3]
Hospital length of stay, days, median [IQR]	6.5[1.7, 11.5]	1.8[1, 5.5]	8.7[7.1, 13]	10.9[7.5, 16.1]

Parameter	All Patients (n = 28)	Mild TBI (n = 14)	Moderate TBI (n =3)	Severe TBI (n =11)
Discharge GCS, median [IQR]	15[15, 15]	14.5[15, 15]	15[14, 15]	15[12, 15]
Discharge disposition				
Home	20(71.4%)	14(100%)	2(66.7%)	4(36.4%)
In-patient Rehabilitation facility	7(25%)	0	1(33.3%)	6(54.5%)
Death	1(3.6%)	0	0	1(9.1%)

Abbreviations:

SD: standard deviation; IQR: interquartile range; CT: computerized tomography

*Mild TBI: Admission GCS: 13–15,

**Moderate TBI: Admission GCS: 9–12,

***Severe TBI: Admission GCS: 3–8 + Indicates mixed lesion on CT

Table 2

Reference values for Plasma Biomarkers in Children (n = 28) with Traumatic Brain Injury

Angiopoietin (AP-2)

AP-2 median(minimum-maximum) pg/ml					
	Overall	**Overall**	**Mild**	**Moderate**	**Severe**
	n = 28	**n = 28**	**n = 14**	**n = 3**	**n = 11**
AP-2		2610.695(1282.355,10392.66)	2409.44(1282.355,4555.11)	2563.66(1488.87,4565.39)	2998.84(1892.22,10392.66)
Age groups					
0–4 years	n=6	3157.425(2137.015,4654.32)	3065.42(2379.65,3751.19)	3564.525(2563.66,4565.39)	3395.6675(2137.015,4654.32)
5–9 years	n=6	1966.21(1488.87,2998.84)	2420.55(1990.59,2850.51)	1488.87(na)	1941.83(1892.22,2998.84)
10–14 years	n=8	1958.18(1282.355,2657.73)	1674.505(1282.355,2439.23)	0	2456.13(2254.53,2657.73)
15–18 years	n=8	4137.63(2899.59,10392.66)	4137.63(2899.59,4555.11)	0	5656.055(3382.03,10392.66)
Sex (male)					
Female	n=10	2103.372(1478.58,3382.03)	1990.59(1478.58,2439.23)	2563.66(na)	2195.773(1941.83,3382.03)
Male	n=18	3286.425(1282.355,10392.66)	2899.59(1282.355,4555.11)	3027.13(1488.87,4565.39)	3574.01(1892.22,10392.66)
Abnormalities on CT head					
Subdural hematoma (SDH) n =14	Isolated n = 2 Mixed n = 12	2501.445(2439.23,2563.66) 3300.85(1478.58,10392.66)	2439.23(na) 3300.85(1478.58,4555.11)	2563.66(na) 0	0 4937.557(,10392.66)
Epidural hematoma (EDH) n = 8	Isolated n = 1 Mixed n = 7	3574.01(na) 2137.015(1502.38,4565.39)	0 2030.16(1502.38,4421.58)	0 4565.39(na)	3574.01(na) 2397.372(2137.015,2657.73)
Subarachnoid hemorrhage (SAH) n = 12	Isolated n = 0 Mixed n = 12	0 3190.435(1502.38,10392.66)	0 3352.095(1502.38,4555.11)	0 0	0 3190.435(2137.015,10392.66)
Intraparenchymal hemorrhage (IPH) n = 4	Isolated n =1 Mixed n = 3	1282.355(na) 4421.58(2998.84,4555.11)	1282.355(na) 4488.345(4421.58,4555.11)	0 0	0 2998.84(na)
Cerebral edema n = 4	Isolated n = 0 Mixed n = 4	0 3140.81(1892.22,10392.66)	0 2899.59(na)	0 0	0 3382.03(1892.22,10392.66)
Cerebral herniation n = 1	Isolated n = 0 Mixed n = 1	0 1892.22(na)	0 0	0 0	0 1892.22(na)
Diffuse axonal injury n =1	isolated n=1 Mixed n = 0	2254.53(na) 0	0 0	0 0	2254.53(na) 0
Skull fracture n = 15	Isolated n = 0 Mixed n=15	0 2137.015(1478.58,10392.66)	0 2069.73(1478.58,4421.58)	0 3027.13(1488.87,4565.39)	0 2567.9275(1941.83,10392.66)
Subgaleal hematoma	Isolated n = 0 Mixed n=13	0 2850.51(1478.58,10392.66)	0 2224.69(1478.58,2899.59)	0 4565.39(na)	0 4018.175(1941.83,10392.66)

AP-2 median(minimum-maximum) pg/ml					
	Overall	**Overall**	**Mild**	**Moderate**	**Severe**
	n = 28	**n = 28**	**n = 14**	**n = 3**	**n = 11**
n = 13					
Pneumocephalus n = 7	Isolated n = 0 Mixed n=7	0 2069.73(1478.58,10392.66)	0 1846.63(1478.58,2069.73)	0 3027.13(1488.87,4565.39)	0 6695.75(2998.84,10392.66)
Intraventricular hemorrhage (IVH) n = 1	Isolated n = 0 Mixed n=1	0 7738.1(na)	0 0	0 0	0 7738.1(na)

Endothelin-1(ET-1)

Endohelin-1 median(minimum-maximum) pg/ml					
	Overall	Overall	Mild	Moderate	Severe
	n = 28	n = 28	n = 14	n = 3	n = 11
ET-1		92.9675(25.86,502.5)	91.945(31.3115,502.5)	87.72(49.34,105.68)	94.565(25.86,126.23)
Age groups					
0–4 years	n=6	101.265(49.34,261.12)	157.0125(52.905,261.12)	77.51(49.34,105.68)	101.265(97.81,104.72)
5–9 years	n=6	100.3275(87.72,502.5)	304.295(106.09,502.5)	87.72(na)	94.565(91.37,126.23)
10–14 years	n=8	97.075(31.3115,176.35)	73.58(31.3115,176.35)	0	103.52(98.37,108.67)
15–18 years	n=8	70.905(25.86,132.55)	85.175(68.08,132.55)	0	63.895(25.86,73.73)
Sex (male)					
Female	n=10	96.795(41.745,502.5)	95.78(41.745,502.5)	49.34(na)	98.09(60.64,126.23)
Male	n=18	89.74(25.86,261.12)	88.11(31.3115,261.12)	96.7(87.72,105.68)	91.37(25.86,108.67)
Abnormalities on CT head					
SDH n =14	Isolated n = 2 Mixed n = 12	72.56(49.34,95.78) 90.025(51.38,261.12)	95.78(na) 94.165(51.38,261.12)	49.34(na) 0	0 85.77(67.15,126.23)
EDH n = 8	Isolated n = 1 Mixed n = 7	25.86(na) 105.68(41.745,502.5)	0 91.965(41.745,502.5)	0 105.68(na)	25.86(na) 103.24(97.81,108.67)
SAH n = 12	Isolated n = 0 Mixed n = 12	0 94.59(51.38,132.55)	0 94.165(51.38,132.55)	0 0	0 94.59(60.64,108.67)
IPH n = 4	Isolated n =1 Mixed n = 3	31.3115(na) 91.37(82.24,132.55)	31.3115(na) 107.395(82.24,132.55)	0 0	0 91.37(na)
Cerebral edema n = 4	Isolated n = 0 Mixed n = 4	0 80.92(60.64,94.565)	0 88.11(na)	0 0	0 73.73(60.64,94.565)
Cerebral herniation n = 1	Isolated n = 0 Mixed n = 1	0 94.565(na)	0 0	0 0	0 94.565(na)

Endohelin-1 median(minimum-maximum) pg/ml					
Diffuse axonal injury n =1	isolated n=1 Mixed n = 0	98.37(na) 0	0 0	0 0	98.37(na) 0
Skull fracture n = 15	Isolated n = 0 Mixed n=15	0 97.81(41.745,502.5)	0 106.09(41.745,502.5)	0 96.7(87.72,105.68)	0 94.59(73.73,126.23)
Subgaleal hematoma n = 13	Isolated n = 0 Mixed n=13	0 97.81(41.745,261.12)	0 97.1(41.745,261.12)	0 105.68(na)	0 85.77(60.64,126.23)
Pneumocephalus n = 7	Isolated n = 0 Mixed n=7	0 91.37(41.745,176.35)	0 122.63(41.745,176.35)	0 96.7(87.72,105.68)	0 82.55(73.73,91.37)
IVH n = 1	Isolated n = 0 Mixed n=1	0 67.15(na)	0 0	0 0	0 67.15(na)

Endocan (EC-2)

EC-2 median(minimum-maximum) pg/ml					
	Overall	**Overall**	**Mild**	**Moderate**	**Severe**
	n = 28	**n = 28**	**n = 14**	**n = 3**	**n = 11**
EC-2		2032.755(511.07,5625.625)	2072.01(511.07,2573.95)	1500.41(1024.84,2125.91)	2011.74(528.79,5625.625)
Age groups					
0–4 years	n=6	1136.685(511.07,2200.9)	1355.985(511.07,2200.9)	1575.375(1024.84,2125.91)	1136.685(1032.58,1240.79)
5–9 years	n=6	1172.355(528.79,2490.365)	1172.355(976.28,1368.43)	1500.41(na)	919.06(528.79,2490.365)
10–14 years	n=8	1998.91(978.53,2573.95)	2019.925(978.53,2573.95)	0	1664.295(1316.85,2011.74)
15–18 years	n=8	2389.375(2090.25,5625.625)	2175.815(2090.25,2305.86)	0	2891.0825(2472.89,5625.625)
Sex (male)					
Female	n=10	1852.568(978.53,2490.365)	1719.055(978.53,2278.45)	2125.91(na)	1894.87(1032.58,2490.365)
Male	n=18	2072.01(511.07,5625.625)	2095.89(511.07,2573.95)	1262.625(1024.84,1500.41)	2011.74(528.79,5625.625)
Abnormalities on CT head					
SDH n =14	Isolated n = 2 Mixed n = 12	2055.995(1986.08,2125.91) 2148.395(511.07,3272.335)	1986.08(na) 2093.07(511.07,2278.45)	2125.91(na) 0	0 2500.0975(1032.58,3272.335)
EDH n = 8	Isolated n = 1 Mixed n = 7	5625.625(na) 1719.055(1024.84,2278.45)	0 1987.3975(1368.43,2278.45)	0 1024.84(na)	5625.625(na) 1522.16(1032.58,2011.74)
SAH n = 12	Isolated n = 0 Mixed n = 12	0 2072.01(528.79,3272.335)	0 2093.07(976.28,2278.45)	0 0	0 1626.265(528.79,3272.335)
IPH n = 4	Isolated n =1 Mixed n = 3	2573.95(na) 2095.89(528.79,2255.74)	2573.95(na) 2175.815(2095.89,2255.74)	0 0	0 528.79(na)
Cerebral edema n = 4	Isolated n = 0 Mixed n = 4	0 2389.375(919.06,3272.335)	0 2305.86(na)	0 0	0 2472.89(919.06,3272.335)

EC-2 median(minimum-maximum) pg/ml					
	Overall	**Overall**	**Mild**	**Moderate**	**Severe**
	n = 28	**n = 28**	**n = 14**	**n = 3**	**n = 11**
Cerebral herniation n = 1	Isolated n = 0 Mixed n = 1	0 919.06(na)	0 0	0 0	0 919.06(na)
Diffuse axonal injury n =1	isolated n=1 Mixed n = 0	1316.85(na) 0	0 0	0 0	1316.85(na) 0
Skull fracture n = 15	Isolated n = 0 Mixed n=15	0 1719.055(528.79,3272.335)	0 2053.77(976.28,2278.45)	0 1262.625(1024.84,1500.41)	0 1761.4725(528.79,3272.335)
Subgaleal hematoma n = 13	Isolated n = 0 Mixed n=13	0 1719.055(511.07,3272.335)	0 1348.7925(511.07,2305.86)	0 1024.84(na)	0 2481.6275(1032.58,3272.335)
Pneumocephalus n = 7	Isolated n = 0 Mixed n=7	0 1500.41(528.79,3272.335)	0 1719.055(978.53,2053.77)	0 1262.625(1024.84,1500.41)	0 1900.5625(528.79,3272.335)
IVH n = 1	Isolated n = 0 Mixed n=1	0 2509.83(na)	0 0	0 0	0 2509.83(na)

Interleukin-6 (IL-6)

IL-6 median(minimum-maximum) pg/ml					
	Overall	**Overall**	**Mild**	**Moderate**	**Severe**
	n = 28	**n = 28**	**n = 14**	**n = 3**	**n = 11**
IL-6		23.3675(6.26,180.54)	23.825(6.26,92.12)	19.42(10.96,22.02)	32.1(11.32,180.54)
Age groups					
0–4 years	n=6	31.07(10.96,95.17)	65.0275(37.935,92.12)	15.19(10.96,19.42)	59.6875(24.205,95.17)
5–9 years	n=6	20.6275(15.68,32.1)	20.84(19.15,22.53)	22.02(na)	19.235(15.68,32.1)
10–14 years	n=8	13.895(6.26,37.645)	10.32(6.26,31.47)	0	29.1775(20.71,37.645)
15–18 years	n=8	36.395(11.32,180.54)	28.105(25.12,43.84)	0	85.81(11.32,180.54)
Sex (male)					
Female	n=10	19.1925(6.26,97.09)	11.71(6.26,31.47)	10.96(na)	57.94(19.235,97.09)
Male	n=18	26.19(7.65,180.54)	27.26(7.65,92.12)	20.72(19.42,22.02)	32.1(11.32,180.54)
Abnormalities on CT head					
SDH n =14	Isolated n = 2 Mixed n = 12	9.945(8.93,10.96) 30.21(11.32,95.17)	8.93(na) 30.21(11.71,92.12)	10.96(na) 0	0 46.88(11.32,95.17)
EDH n = 8	Isolated n = 1 Mixed n = 7	180.54(na) 19.42(6.26,95.17)	0 15.43(6.26,25.12)	0 19.42(na)	180.54(na) 66.408(37.645,95.17)

IL-6 median(minimum-maximum) pg/ml					
	Overall	**Overall**	**Mild**	**Moderate**	**Severe**
	n = 28	**n = 28**	**n = 14**	**n = 3**	**n = 11**
SAH n = 12	Isolated n = 0 Mixed n = 12	0 27.035(11.71,97.09)	0 23.825(11.71,43.84)	0 0	0 56.09(15.68,97.09)
IPH n = 4	Isolated n =1 Mixed n = 3	7.65(na) 25.12(15.68,43.84)	7.65(na) 34.48(25.12,43.84)	0 0	0 15.68(na)
Cerebral edema n = 4	Isolated n = 0 Mixed n = 4	0 53.315(27.26,97.09)	0 27.26(na)	0 0	0 74.53(32.1,97.09)
Cerebral herniation n = 1	Isolated n = 0 Mixed n = 1	0 32.1 (na)	0 0	0 0	0 32.1 (na)
Diffuse axonal injury n =1	isolated n=1 Mixed n = 0	20.71(na) 0	0 0	0 0	20.71(na) 0
Skull fracture n = 15	Isolated n = 0 Mixed n=15	0 22.02(6.26,95.17)	0 22.53(6.26,37.935)	0 20.72(19.42,22.02)	0 46.8825(15.68,95.17)
Subgaleal hematoma n = 13	Isolated n = 0 Mixed n=13	0 24.205(6.26,97.09)	0 24.895(6.26,92.12)	0 19.42(na)	0 49.3675(11.32,97.09)
Pneumocephalus n = 7	Isolated n = 0 Mixed n=7	0 19.42(6.26,74.53)	0 16.08(6.26,31.47)	0 20.72(19.42,22.02)	0 45.105(15.68,74.53)
IVH n = 1	Isolated n = 0 Mixed n=1	0 11.32(na)	0 0	0 0	0 11.32(na)

Median (minimum – maximum) level calculated for each biomarker

Median (minimum – maximum) level calculated separately for isolated or mixed lesion on CT (na) indicates only one case included in analysis

Table 3

Association between Plasma Biomarkers and Cerebral autoregulation in Hospitalized Children (n = 25) with Traumatic Brain Injury

	Angiopoietin-2*		Endothelin-1*		Endocan-2*		Interleukin-6*	
	Coefficient	**95% CI**	**Coefficient**	**95% CI**	**Coefficient**	**95% CI**	**Coefficient**	**95% CI**
Cerebral autoregulation								
Normal		*Reference*		*Reference*		*Reference*		*Reference*
Impaired	350.63	(−1498.11, 2199.38)	−31.02	(−118.62, 56.57)	−316.66	(−1304.84, 671.51)	−13.29	(−48.98, 22.39)
TBI severity								
Mild		*Reference*		*Reference*		*Reference*		*Reference*
Moderate	−950.67	(−5407.32, 3505.99)	−58.42	(−269.59, 152.75)	−413.99	(−2796.14, 1968.14)	−10.02	(−96.04, 75.99)
Severe	1271.21	(−577.53, 3119.95)	−37.29	(−124.89, 50.3)	445.06	(−543.12, 1433.24)	32.92	(−2.76, 68.6)

*Values expressed as pg/ml