Autologous Bone Marrow Mononuclear Cells Reduce Therapeutic Intensity for Severe Traumatic Brain Injury in Children*

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Objectives: The devastating effect of traumatic brain injury is exacerbated by an acute secondary neuroinflammatory response, clinically manifest as elevated intracranial pressure due to cerebral edema. The treatment effect of cell-based therapies in the acute post–traumatic brain injury period has not been clinically studied although preclinical data demonstrate that bone marrow–derived mononuclear cell infusion down-regulates the inflammatory response. Our study evaluates whether pediatric traumatic brain injury patients receiving IV autologous bone marrow–derived mononuclear cells within 48 hours of injury experienced a reduction in therapeutic intensity directed toward managing elevated intracranial pressure relative to matched controls.

Design: The study was a retrospective cohort design comparing pediatric patients in a phase I clinical trial treated with IV autologous bone marrow–derived mononuclear cells ($n = 10$) to a control group of age- and severity-matched children ($n = 19$).

Setting: The study setting was at Children's Memorial Hermann Hospital, an American College of Surgeons Level 1 Pediatric Trauma Center and teaching hospital for the University of Texas Health Science Center at Houston from 2000 to 2008.

Patients: Study patients were 5–14 years with postresuscitation Glasgow Coma Scale scores of 5–8.

Interventions: The treatment group received 6 million autologous bone marrow–derived mononuclear cells/kg body weight IV within 48 hours of injury. The control group was treated in an identical fashion, per standard of care, guided by our traumatic brain injury management protocol, derived from American Association of Neurological Surgeons guidelines.

Measurements and Main Results: The primary measure was the Pediatric Intensity Level of Therapy scale used to quantify treatment of elevated intracranial pressure. Secondary measures included the Pediatric Logistic Organ Dysfunction score and days of intracranial pressure monitoring as a surrogate for length of neurointensive care. A repeated-measure mixed model with marginal linear predictions identified a significant reduction in the Pediatric Intensity Level of Therapy score beginning at 24 hours posttreatment through week 1 ($p < 0.05$). This divergence was also reflected in the Pediatric Logistic Organ Dysfunction score following the first week. The duration of intracranial pressure monitoring was 8.2 ± 1.3 days in the treated group and 15.6 ± 3.5 days ($p = 0.03$) in the time-matched control group.

Conclusions: IV autologous bone marrow–derived mononuclear cell therapy is associated with lower treatment intensity required to manage intracranial pressure, associated severity of organ injury, and duration of neurointensive care following severe traumatic brain injury. This may corroborate preclinical data that autologous bone marrow–derived mononuclear cell therapy attenuates the effects of inflammation in the early post–traumatic brain injury period. (*Pediatr Crit Care Med* 2015; 16:245–255)

*See also p. 294.

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Traumatic brain injury (TBI) continues to present a profound financial and social burden to the population due to its associated morbidity and disability (1, 2). Despite improvements in prevention, severe pediatric TBI, defined clinically as a Glasgow Coma Score (GCS) less than 8, rates remain unchanged (3).

TBI causes an acute secondary neuroinflammatory response clinically manifest as elevated intracranial pressures (ICPs) from cerebral edema. The pathophysiology and management of TBI can be viewed in two stages (4). The first stage is treating the primary injury and the sequela of the direct mechanical impact and involves evacuating gross macrovascular bleeding, achieving hemostasis and debridement

TABLE 1. Pediatric Intensity Level of Therapy Scale

Variable	Score	Maximum Possible Score
General—occurring at any time in 24-hr period		
Treatment of fever (temperature of > 38.5°C) or spontaneous temperature of < 34.5°C	1	4
Sedation (e.g., narcotics, benzodiazepines: any dose)	1	
Neuromuscular blockade	2	
Ventilation—most frequently observed $Paco_2$ in 24-hr period		
Intubated/normal ventilation ($Paco_2$ of 35.1–40 mm Hg)	1	4
Mild hyperventilation ($Paco_2$ of 32–35 mm Hg)	2	
Aggressive hyperventilation ($Paco_2$ of < 32 mm Hg)	4	
Osmolar therapy—total dose in 24-hr period		
Mannitol, ≤ 1 g/kg	1	6
Mannitol, 1.1–2 g/kg	2	
Mannitol, > 2 g/kg	3	
or		
Hypertonic saline (any dose or rate, regardless of serum [Na])	3	
Cerebrospinal fluid drainage—number of times in 24-hr period		
0–11 times	1	3
12–23 times	2	
≥ 24 times or continuous	3	
Barbiturates—total dose in 24-hr period		
Pentobarbital, ≤ 36 mg/kg	3	4
Pentobarbital, > 36 mg/kg	4	
Surgery—at any time in 24-hr period		
Evacuation of hematoma	4	9
Decompressive craniectomy	5	
Other—at any time during 24-hr period		
Induced hypothermia		8
Mild (≥ 35°C to 37°C)	2	
Moderate (< 35°C)	4	
Lumbar drain	2	
Induced hypertension (≥ 95th percentile for age)	2	
Total possible score	38	

TABLE 2. Clinical Trial Inclusion/Exclusion Criteria

Inclusion criteria
Age, 5–14 yr
Postresuscitation Glasgow Coma Score, 5–8
Injury occurring < 24 hr within enrollment
Exclusion criteria
Initial intracranial pressure > 40
Findings on head CT/MRI suggestive of prolonged hypoxic ischemic insult
Hemodynamic instability
Uncorrected coagulopathy at the time of harvest
Pulmonary contusions
Solid or hollow visceral injury of the abdomen/pelvis
Spinal cord injury

of nonviable tissue. The second and often long-term goal is directed toward treating the secondary effects of the initial impact, which involves a neuroinflammatory response exacerbated by the breakdown of the blood-brain barrier, resulting in subacute, life-threatening cerebral edema. This second stage classically peaks approximately 48–72 hours after the initial trauma (5–7). Subsequent chronic inflammation and cellular dysfunction manifest as chronic motor and cognitive disabilities.

The acute neurointensive care of TBI has unfortunately remained supportive and focuses on treating edema and escalates in intensity in a tiered fashion (8, 9). First-tier treatments include establishing an ICP treatment threshold, cerebral perfusion pressure (CPP) monitoring, sedation, neuromuscular blockade, cerebrospinal fluid (CSF) drainage, and hyperosmolar therapy with mannitol or 3% saline (10). Second-tier recommendations include hyperventilation, barbiturates, hypothermia, and decompressive craniotomy (11–24). In the pediatric population, the Pediatric Intensity Level of Therapy (PILOT) scoring system for treatment intensity directed toward ICP management has been developed and validated with higher scores assigned to second-tier management strategies such as hyperventilation, barbiturates, hypothermia, and decompressive craniectomy (25–28) (**Table 1**).

ICP is monitored and used either as a therapeutic target (< 25 mm Hg) or as a component in a CPP strategy (CPP = mean arterial pressure – ICP). After excluding extra-axial bleeding and contusion expansion with imaging studies, the ICP is functionally used as a surrogate for cerebral edema. Cerebral edema has been categorized as vasogenic (interstitial) and cytotoxic (intracellular), but both are consequences of the secondary brain injury that could be exacerbated by neuroinflammation (29–31). Sustained elevations of ICP suggest ongoing secondary injury. Elevated average ICP in the first 48 hours of monitoring has been shown to be independently associated with mortality (32). Despite ICP-targeted therapy, one third of pediatric patients with severe TBI have unfavorable outcomes: death, persistent vegetative state, or severe/moderate disability (1, 3).

Our translational research laboratory has developed a focused effort investigating preclinical and clinical applications of cell therapy (33). Bone marrow–derived mononuclear cells (BMMNCs) comprise a progenitor cell population that share the characteristics of unilobulated or round nuclei, absence of granules in the cytoplasm, and similar size and density which allows for easy isolation for therapeutic application (34) These cells have been shown to mobilize in response to tissue damage to organs such as the heart, liver, and kidneys (35–37). In 2008,

TABLE 3. Baseline Demographic/Injury Data for Phase I and Control Cohorts

Variable	Control 2000–2006 ($n = 14 \pm$ SEM)	Control 2006–2008 ($n = 5 \pm$ SEM)	p (2000–2006 vs 2006–2008)	Control 2000–2008 ($n = 19 \pm$ SEM)	Phase I (2006–2008) ($n = 10 \pm$ SEM)	p (Control 2006–2008 vs Phase I)	p (Control 2000–2008 vs Phase I)
Males, %	64 ($n = 9$)	40 ($n = 2$)	0.6	60 ($n = 11$)	70 ($n = 7$)	0.3	0.7
Mean age	8.9 ± 0.7	8.6 ± 1.6	0.8	8.8 ± 0.8	8.9 ± 0.9	0.9	1.0
Injury Severity Score	29 ± 1.1	35 ± 6.6	0.03	30 ± 1.5	30 ± 1.9	0.2	0.3
Best initial Glasgow Coma Score	6.3 ± 0.3	6.6 ± 1.1	0.7	6.4 ± 0.4	5.8 ± 0.4	0.3	0.3
Modified Marshall Score	3.7 ± 0.3	3.3 ± 1.7	0.6	3.6 ± 0.3	3.7 ± 0.5	0.7	0.8
Opening pressure[a]	20 ± 2.7 ($n = 12$)	22 ± 4.3 ($n = 5$)	0.7	22 ± 2.4 ($n = 17$)	21 ± 2.1 ($n = 10$)	0.6	0.7
Craniectomies, %	29 ($n = 4$)	20 ($n = 1$)	0.1	26 ($n = 5$)	50 ($n = 10$)	0.1	0.4
External ventricular drain, %	42 ($n = 5$)	0	0.2	29 ($n = 5$)	40 ($n = 4$)	0.1	0.5

[a]Two patients in the 2000–2006 control group did not have intracranial pressure monitors placed.

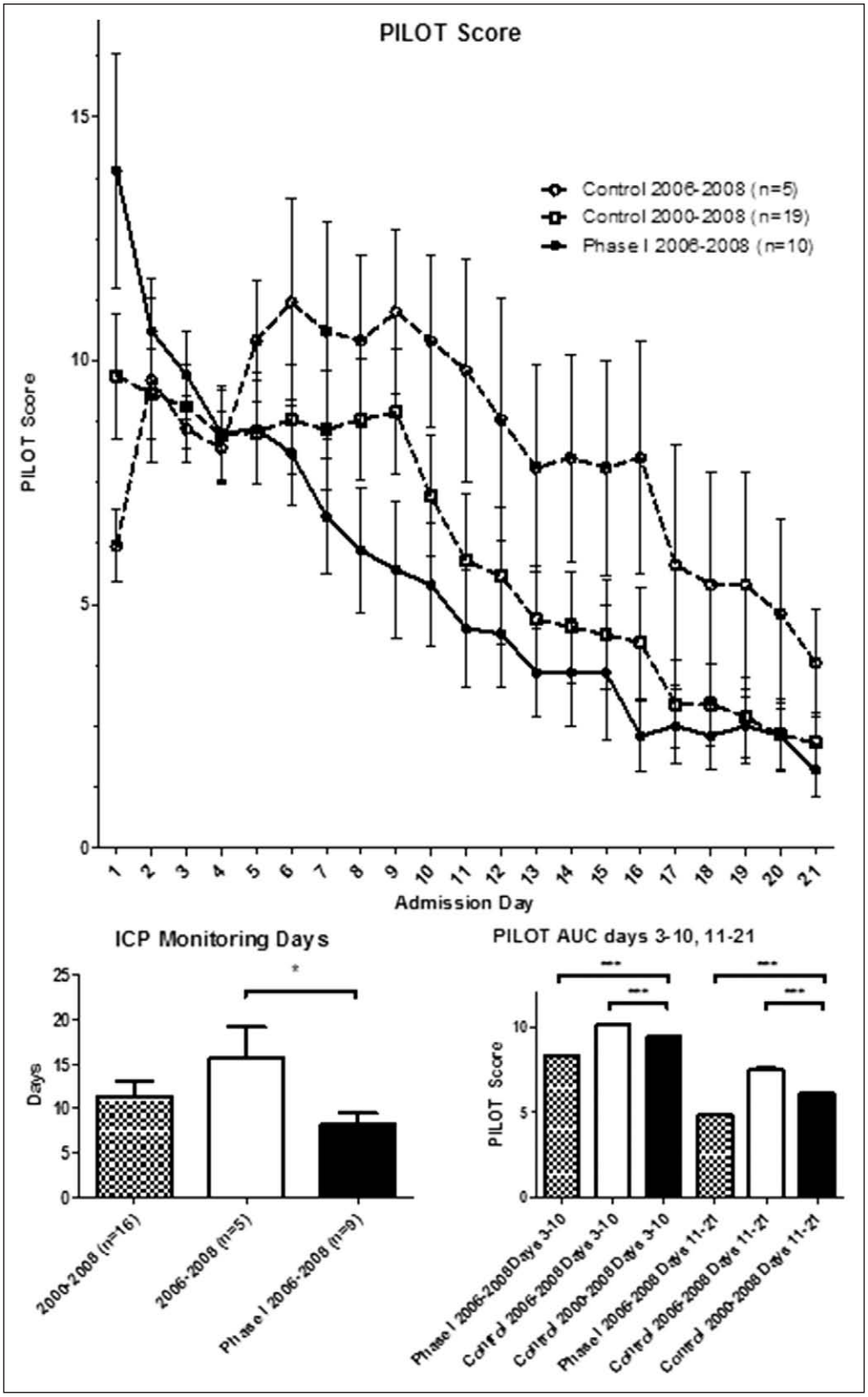

Figure 1. Pediatric Intensity Level of Therapy (PILOT) score calculated from time of admission with divergence seen following time of cell therapy (patients were treated within 48 hr of admission) with $p < 0.001$ versus both 2006–2008 and 2000–2008 controls. Also displayed are the mean intracranial pressure (ICP) monitoring days with SEM for each group and area under the curve (AUC) analysis where cumulative PILOT scores in each group of patients were summed for days 3–10 and for days 11–21.

we completed a 2-year phase I trial with 10 children using autologous BMMNCs delivered IV within 48 hours of injury and found this approach to be safe, although a secondary finding with underlying mechanistic potential was preservation of selected brain structures on follow-up MRI (38). Concurrent preclinical rodent experiments demonstrated that cell-based therapies could reduce the amount of locoregional brain edema in the acute postinjury period, suggesting that therapy allows for preservation or recovery of the blood-brain barrier (39–41). We thus questioned whether or not this preclinical acute treatment-associated effect could be retrospectively demonstrated in our human trials. In this study, we sought to evaluate whether our previous cohort of treated pediatric TBI patients experienced a reduction in ICP-directed therapeutic intensity levels relative to time, age, and severity-matched control patients.

MATERIALS AND METHODS

This study was a retrospective cohort study using data obtained from a clinical trial conducted under Federal Investigational New Drug Application BB 12620 and was approved by The University of Texas Health Sciences Center at Houston Committee for the Protection of Human Subjects and approved by the Children's Memorial Hermann Office of Research.

All 10 patients who were enrolled in our phase I trial (May 2006–October 2008) were included in this cohort study as the treatment group. These patients were reported in a previous publication (38).

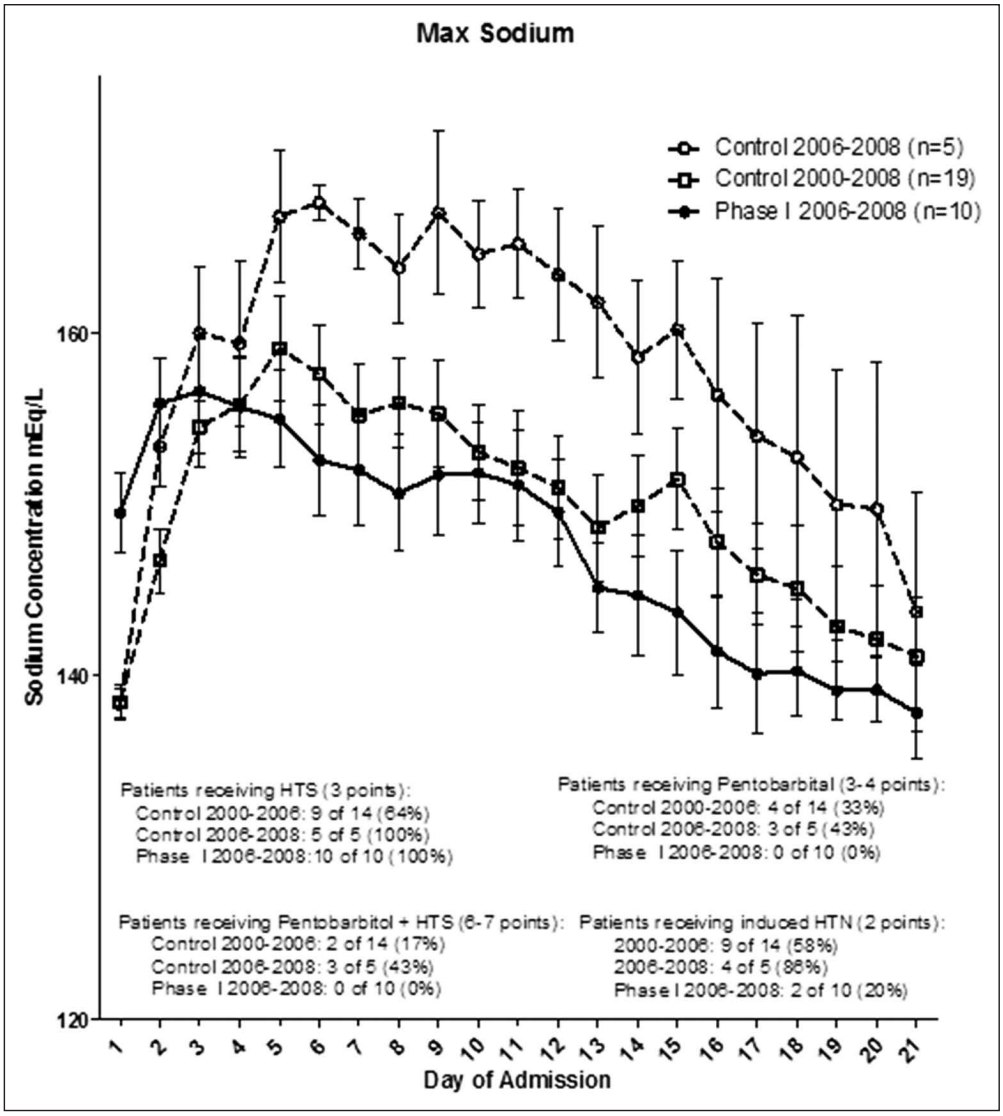

Figure 2. Maximum sodium levels were highest in the 2006–2008 control group, which were significantly higher than the 2006–2008 phase I group ($p < 0.05$). HTN = hypertension, HTS = hypertonic saline.

A control group of 19 patients was formed by applying the same inclusion/exclusion criteria (**Table 2**) to 156 consecutive severe TBI children (defined as GCS ≤ 8) who were admitted to our same institution from the years 2000 to 2008. Five of the 19 patients who were admitted during the phase I trial period were not enrolled. Three of these patients had declined to be included in the trial and two were not enrolled because their treatment window would have conflicted with the safety monitoring review period for one of the enrolled phase I trial patients. Age, sex, Injury Severity Score, best initial GCS, Modified Marshall Score, and opening ICP were used for baseline characteristics (**Table 3**). Opening pressure data were only available for patients from 2006 to 2008. Two-tailed *t* tests and Fisher exact tests were used to compare the two groups at baseline. Means were reported with SEM.

Bone marrow at 3–5 mL/kg body weight was harvested aseptically from either the posterior iliac bone or the anterior iliac crests while under both local and systemic anesthesia and with continuous physiologic monitoring. Once collected, the marrow was processed by the Center for Cell and Gene Therapy. In brief, the filtered mononuclear cell fraction was isolated using Ficoll-Paque PLUS (GE Healthcare Bio-Sciences, Piscataway, NJ) density gradient separation. The mononuclear cells were washed with human serum albumin in normal saline and adjusted to the appropriate concentration of 6×10^6 BMMNC/kg at a volume of 1 cc/kg of body weight. Prior to release, the cells were tested for viability and presence of endotoxin. Quality control was conducted for bacterial and fungal cultures, presence of mycoplasma via polymerase chain reaction as well as progenitor cell colony formation, and flow cytometry for cell characteristics. The product was infused IV using catheters no smaller than 20 gauge.

Treatment Intensity–PILOT Score

The primary measure of this study was the intensity of treatment required to counter elevated ICP due to the neuroinflammatory response to injury. The PILOT score was calculated at 24-hour intervals starting from the time of admission, by combining subtotals from seven different treatment modalities (**Table 1**). A repeated-measure mixed model with marginal linear predictions was used to identify if there were any differences between treatment and control groups from the time of admission through 21 days as well as from hospital day 3 to 10 (24 hr after infusion of cell product for the treatment group). An area under the curve analysis was also conducted to determine if treated patients had less intense cumulative therapy over the first and or second week compared to controls. The PILOT score does not account for different degrees of daily hypertonic saline administration, thus peak serum sodium levels were also compared between groups.

Pediatric Logistic Organ Dysfunction Score

The Pediatric Logistic Organ Dysfunction (PELOD) score is a prospectively validated outcomes measure of the degree of multiple organ dysfunction in pediatric patients (42–47). The PELOD score was calculated daily from 12 variables derived

from six organ system categories: neurological, cardiovascular, renal, respiratory, hematological, and hepatic. For each variable, the most abnormal daily variable was used. A separate comparison between groups was conducted for the GCS, a major component of the PELOD score for TBI patients.

ICP Days

Our study was designed to study the effects of cell-based therapy on TBI, and thus, the inclusion and exclusion criteria were designed to allow enrollment of patients without major organ system dysfunctions at the time of admission. Rather than using the length of stay as an indicator of short-term outcome, we used ICP monitoring days as a more specific surrogate of duration of neurointensive treatment required. In the control group, one patient was excluded from ICP monitor days due to death, one patient was managed without invasive monitoring, and one patient underwent craniectomy and the neurosurgeon elected to not leave a pressure monitor. In the treated group, one patient developed hydrocephalus and underwent multiple ventriculostomies and creation of a ventriculoperitoneal shunt. All groups demonstrated normal distribution via the Shapiro-Wilk and D'Agostino-Pearson omnibus normality tests.

RESULTS

There was no statistically significant difference in baseline characteristics between the treated phase I group and the control groups (**Table 3**). In the 21 days following injury, there were no deaths in the treatment group and one death in the control group.

Treatment Intensity (PILOT)

The treated group experienced a statistically significant reduction in PILOT scores beginning at 24 hours posttreatment through week 1 ($p < 0.05$) (**Fig. 1**). The divergence of the PILOT scores began approximately 48 hours from the time of admission, which corresponded to the period in which treated patients received autologous bone marrow mononuclear cells. Following cell therapy, the divergence in the PILOT score tracing was maintained, with the treatment group following a near linear decline through 21 days. In the first week, the control group treatment intensity remained elevated while the treated patients experienced a de-escalation in treatment intensity following cell therapy. The control group required equal or escalated therapy and did not approach the treatment group scores until after 2 weeks post injury. All phase I patients ($n = 10$) received hypertonic therapy and reached a peak serum sodium concentration of 160 ± 3 mEq/L. This was not statistically significant when compared to nine of 19 patients in the control group that underwent hypertonic saline therapy (163 ± 3.0 mEq/L; $p = 0.5$). However, there was statistical difference between the time-matched subgroup of 2006–2008 at 170 ± 3 mEq/L and the phase I treated group ($p = 0.02$) (**Fig. 2**).

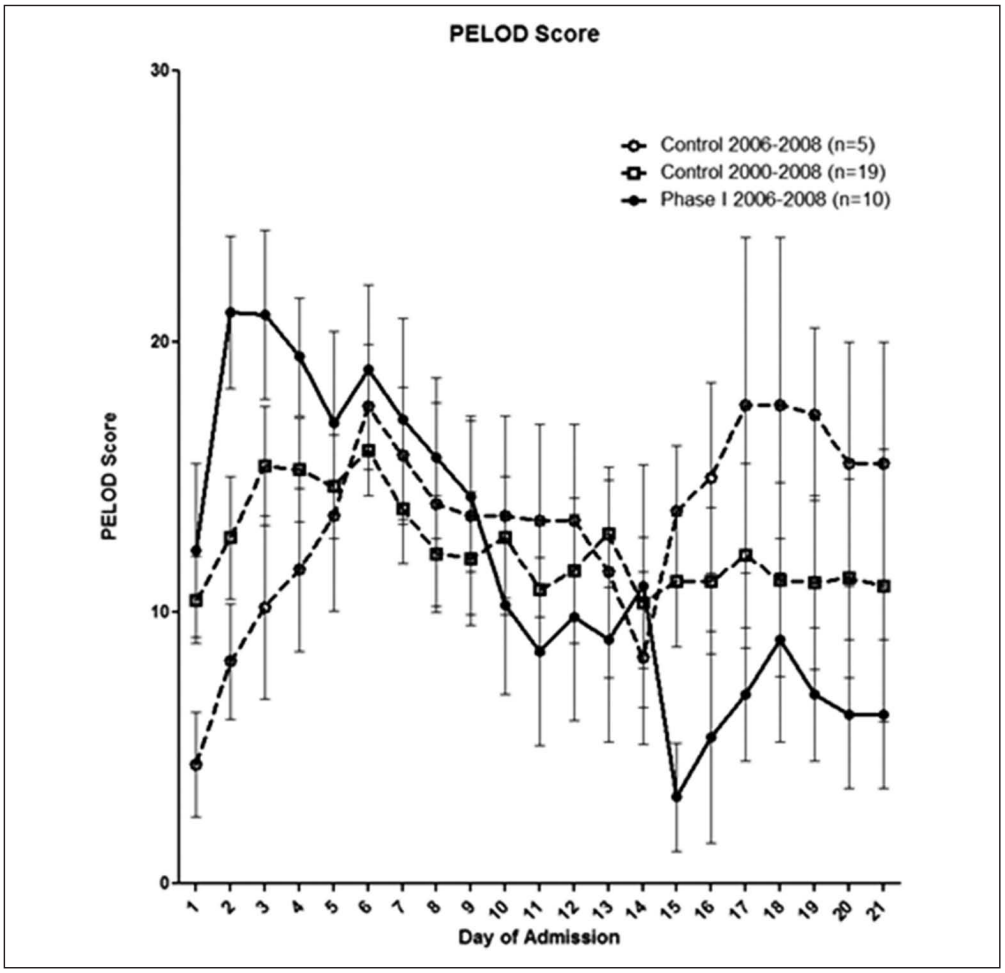

Figure 3. Pediatric Level of Organ Dysfunction (PELOD) score calculated from time of admission with divergence seen after 1 wk with the phase I 2006–2008 group versus both 2006–2008 and 2000–2008 controls ($p < 0.001$).

Organ Dysfunction (PELOD)

The divergence seen in the PILOT score was also reflected in the PELOD score (**Fig. 3**) but appeared to occur at 1 week compared to the first 24–48 hours seen in the PILOT score. GCS also began to improve beginning at 1 week in the treated group (**Fig. 4**).

ICP Monitoring

Maximum ICP levels per day begin to decrease approximately 48 hours after treatment in phase I patients (**Fig. 5**). The mean duration of ICP monitoring was 8.2 ± 1.3 days in

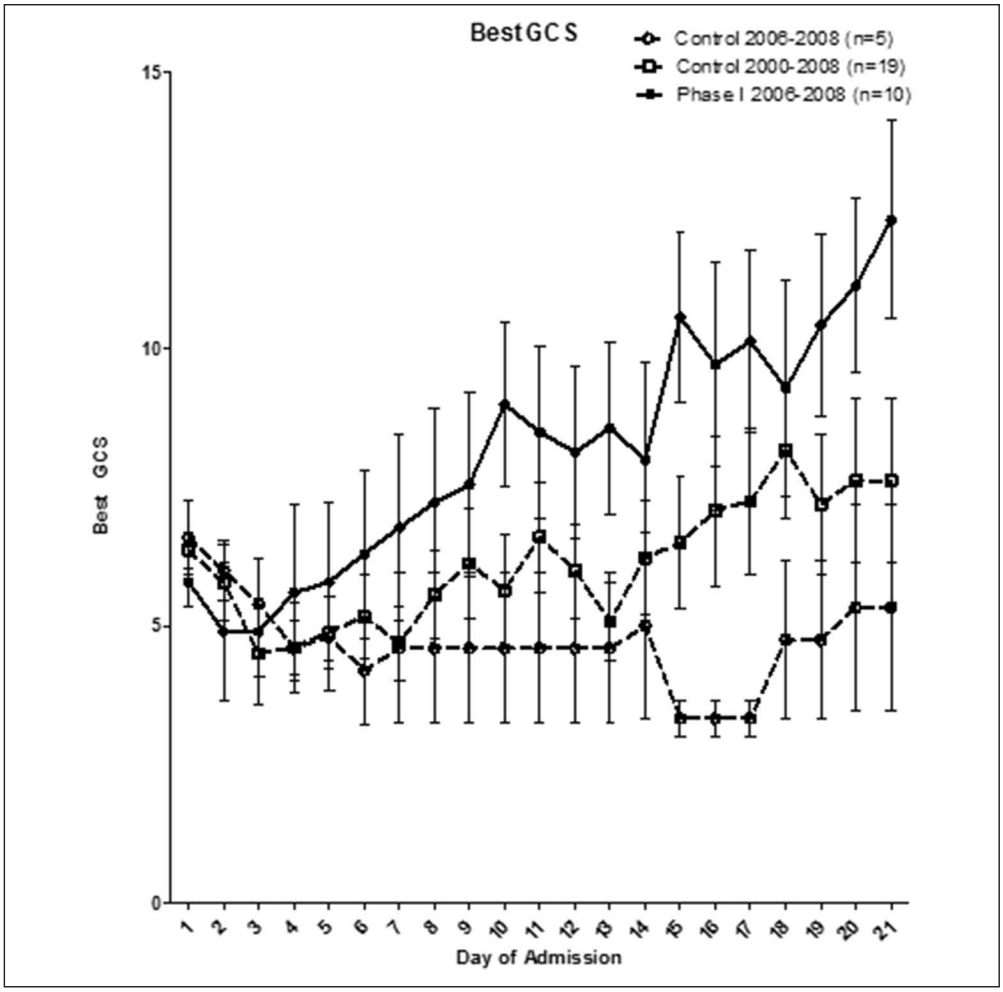

Figure 4. When plotting best Glasgow Coma Score (GCS) per day, there appears to be a cell therapy–related improvement in GCS after 1 wk in the phase I 2006–2008 patients compared to either control group.

the treated group, which was significantly less compared to 15.6 ± 3.5 days in the time-matched control group ($p = 0.03$), but not significantly less when compared to the entire control group 11 ± 1.8 days ($p = 0.2$).

DISCUSSION

Our retrospective cohort study suggests that IV infusion of BMMNCs is associated with lower treatment intensity levels in pediatric patients with severe TBI in the early postinjury period. The divergence of clinical status as defined by the PILOT for treatment intensity and PELOD score for organ dysfunction occurs at distinct time periods. For the PILOT score, the point of divergence, which occurs at the time of autologous bone marrow mononuclear cell infusion, suggests that BMMNCs may be exerting an immediate down-regulatory effect on factors contributing to cerebral edema. Furthermore, the divergence is sustained, suggesting that the cellular therapy exerts a continued primary or propagative effect that is durable up to 2 weeks. Together with the duration of ICP monitoring, the PELOD data suggest that the BMMNCs reduce organ dysfunction past the time of neurointensive care and that the sustained elevated PELOD scores in the control group were more attributed to sustained low GCS from managing ICP/edema or encephalopathy rather than organ dysfunction such as known pulmonary complications associated with TBI (48, 49). In the BMMNC-treated group, the GCS begin to improve at 1 week, the same time the PELOD scores begin to improve, suggesting that the GCS is an important contributor to the PELOD score.

The heterogeneity in TBI injury patterns makes short- and long-term outcome trials very difficult to conduct and interpret (28). Our retrospective study has several limitations. First, this study used a relatively small retrospective cohort design. The phase I trial used in this study as the treatment group only had 10 patients. The phase I trial was also not blinded. The control group included patients from a time period both prior to and during the safety trial. However, due to the nature of the phase I trial, only five of the 19 control patients were admitted within the same period of the phase I trial. Although all patients in this study were from the same institution and selected consecutively, specific treatment strategies used by different clinicians from 2000 to 2008 were not captured by our data analysis. Dean et al (50) reported in 2007 that neurointensivists have least agreement in serum osmolality thresholds for hypertonic therapy, prophylactic hyperventilation, and ICP thresholds. Second, the injury pattern of TBI is very heterogeneous, making the initial injury severity classification difficult. Therefore, in addition to initial GCS, we also used the Modified Marshall CT score as well as the opening CSF/ICP to estimate the severity of the initial TBI in our patients (51). The transition from paper charting to electronic medical records also occurred during the study period of 2000–2008, making data gathering such as opening pressure determination difficult. Data were not available to directly compare long-term outcome data between the treatment and control groups. The phase I trial followed up each subject to 6 months post injury.

In this study, the PILOT score was used to describe the intensity of treatment directed toward ICP, a surrogate measure of cerebral edema. The PILOT score has limitations, one

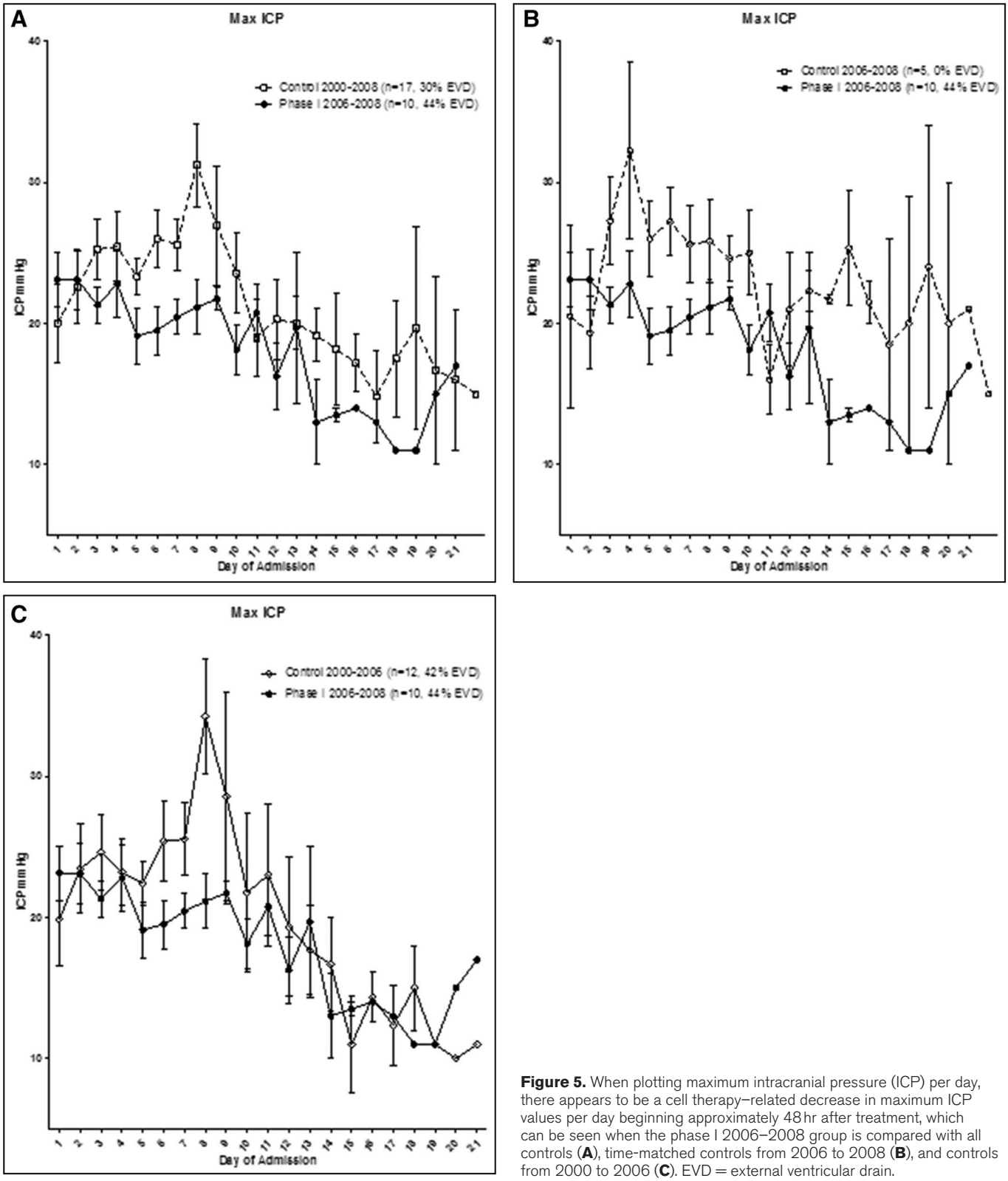

Figure 5. When plotting maximum intracranial pressure (ICP) per day, there appears to be a cell therapy–related decrease in maximum ICP values per day beginning approximately 48 hr after treatment, which can be seen when the phase I 2006–2008 group is compared with all controls (**A**), time-matched controls from 2006 to 2008 (**B**), and controls from 2000 to 2006 (**C**). EVD = external ventricular drain.

of which is in hyperosmolar therapy. The goal serum sodium concentrations are not specified in the scoring system, so patients treated with hypertonic saline to achieve a serum concentration of 148 mEq/L would be scored the same as patients titrated to a serum concentration of 160 mEq/L (52–54). In our study, all of the 10 treatment group patients underwent hypertonic saline therapy and reached a serum sodium concentration of 160 ± 3 mEq/L. This was statistically less than 170 ± 3 mEq/L in the 2006–2008 time-matched control subgroup. When plotting maximum ICP per day, there appears to

be a cell therapy–related decrease in maximum ICP values per day beginning approximately 48 hours after treatment. Also patients in the phase I clinical trial required pentobarbital. These differences may be associated with a therapeutic effect of the cell therapy.

Although not statistically significant, the number of patients that received external ventricular drains (EVDs) was not equal across the groups. Forty-two percent of the control patients from 2000 to 2006 had EVDs placed, which if used for CSF drainage may increase the PILOT score by up to 3 points. None of the control patients from 2006 to 2008 underwent EVD placement versus 42% in the 2000–2006 control group ($p = 0.4$). Forty percent of the phase I patients received EVDs ($p = 0.68$ vs 2000–2008). Decompressive craniectomy can alter the course of neurointensive care. Although fewer patients underwent decompressive craniectomy in the control group (26%) compared to treatment group (50%), this was not statistically significant with a Fisher exact statistic p value of 0.42. The effect of decompression on subsequent treatment intensity may need to be further investigated. Excluding patients who underwent craniectomy from both groups did not alter the degree of divergence but did shift the start of divergence to 7 days rather than the first day. Decompressive craniectomy is a component of the PILOT score, so excluding craniectomy patients form the analysis changes the validity of the treatment intensity estimates of the scoring system used in this study.

Although we now have human clinical trial evidence from this study showing decreased treatment intensity as well as preclinical data demonstrating blood-brain barrier permeability preservation and increased proinflammatory microglial apoptosis, the question remains whether clinical and preclinical data can be linked by neuro/systemic cytokines and biomarkers. The correlation of biomarker profiles with treatment intensity would further strengthen our clinical observations. In our current prospective Adult Phase IIA (ClinicalTrials.gov NCT01575470) and Pediatric Phase IIB (ClinicalTrials.gov NCT01851083) studies involving autologous bone marrow mononuclear cell therapy, CSF and peripheral blood are being collected for biomarker analysis of interleukin (IL)-1, IL-2, IL-4, IL-6, IL-8, and tumor necrosis factor-α in both treated and control patients.

Our clinical trial experience in pediatric and adult patients suggests that differences likely exist in the pathophysiology of TBI, and our clinical findings of treatment intensity in the pediatric population may not be identical in our adult population. For example, children are more likely to be coagulopathic in the acute to subacute post injury period compared to adults. Both adult and pediatric patients have been observed to have an initial decline in platelet numbers followed by a rebound effect. This effect appears to be mediated by circulating endothelial progenitor cells and may be modifiable by the treatment effect exerted by our clinical trial protocol (55). In adults, the rebound effect can sometimes be dramatic, with platelet levels exceeding three times the admission level.

Lastly, this retrospective cohort study design was not able to address long-term functional and neurocognitive outcomes between the treatment and control groups. In the treatment group, 40% of patients had an improvement from a Glasgow Outcome Score range of 1–3 to 4–5 between 30 and 180 days post injury. In the control groups, follow-up information was not always available, but at least 35% of patients from 2000 to 2006 (four unknown) and at least one patient from 2006 to 2008 (four unknown) were estimated to have the same outcome improvement from 30 days to beyond 180 days. The pediatric phase IIB study has been designed to address this issue and includes a control group with robust randomization and blinding to help detect functional and neurocognitive treatment effects.

CONCLUSIONS

IV autologous BMMNC therapy was associated with a reduction in treatment intensity required to manage ICP, associated severity of organ injury, and duration of ICP monitoring following severe TBI. This corroborates preclinical data that autologous BMMNC therapy attenuates the effects of inflammation in the early post-TBI period.

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