

# Humoral Factors Enhance Fracture-Healing and Callus Formation in Patients with Traumatic Brain Injury

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**Background:** Scientific evidence is mounting for an association between traumatic brain injury and enhanced osteogenesis. The aim of this study was to correlate the in vitro osteoinductive potential of serum with the features of fracture-healing and the extent of brain damage in patients with severe traumatic brain injury and bone fracture.

**Methods:** Patients with a long-bone fracture and a traumatic brain injury (seventeen patients) or without a brain injury (twenty-four patients) were recruited. The Glasgow Coma Scale score was determined on admission. Radiographs of the fracture were made before surgery, at six weeks, and at three, six, and twelve months after surgery. The time to union was estimated clinically and radiographically, and the callus ratio to shaft diameter was calculated. Serum samples were collected at six, twenty-four, seventy-two, and 168 hours after injury, and their osteogenic potential was determined by measurement of the in vitro proliferation rate of the human fetal osteoblastic cell line hFOB1.19.

**Results:** Patients with a traumatic brain injury had a twofold shorter time to union ( $p = 0.01$ ), a 37% to 50% increased callus ratio ( $p < 0.01$ ), and their sera induced a higher proliferation rate in hFOB cells ( $p < 0.05$ ). A linear relationship was revealed between hFOB cell proliferation rates and the amount of callus formed ( $p < 0.05$ ). The Glasgow Coma Scale score was correlated with the callus ratio on both radiographic projections ( $p < 0.05$ ), time to union ( $p = 0.04$ ), and the proliferation rate of hFOB cells at six hours after injury ( $p = 0.03$ ).

**Conclusions:** Patients with a severe brain injury release unknown humoral factors into the blood circulation that enhance and accelerate fracture-healing.

The existence of an association between traumatic brain injury and enhanced osteogenesis has been debated. Patients who have sustained a severe traumatic brain injury commonly demonstrate alterations in the normal process of bone-healing. A shorter time to bone union with hypertrophic callus formation has been reported in patients with a fracture and an associated severe head injury<sup>1</sup>. Many descriptions of this phenomenon have been reported anecdotally. The most considerable communications include two studies published twenty years ago<sup>2,3</sup>. Those studies documented increased callus formation and a shorter time to union in patients with a severe brain injury and an associated long-bone fracture.

Previous studies have used in vitro cellular models to investigate the pathophysiologic mechanisms underlying these osteogenic phenomena in patients with traumatic brain injury. Boes et al.<sup>4</sup> showed that in vitro human stromal stem cells proliferated beyond expected parameters when incubated with serum from rats with a brain injury, and they concluded that brain-derived factors mediate a mitogenic effect on osteoprogenitor cells by means of the humoral circulation. In earlier studies, Bidner et al.<sup>5</sup>, using osteoblastic cells isolated from the calvaria of fetal rats, demonstrated similar results with serum from patients with a traumatic brain injury. The well-established human fetal osteoblast cell line hFOB1.19 (hFOB) has been described as an early-stage osteoprogenitor cell line<sup>6,7</sup>. This

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cell line has been used successfully in *in vitro* proliferation assays to screen serum and cerebrospinal fluid samples for their osteoinductive potential and, therefore, the presence of osteogenic factors in patients with traumatic brain injury and an associated fracture<sup>8,9</sup>. However, no study to date, as far as we know, has directly provided evidence linking the osteoinductive potential of human serum with use of *in vitro* cellular assays with the clinical features of enhanced callus formation and fracture-healing in patients with traumatic brain injury.

The present study tested the hypothesis that serum from patients with a traumatic brain injury, collected as early as six hours after the injury, would have a greater proliferative influence on the osteoprogenitor cell line hFOB1.19 than would samples from patients without a traumatic brain injury. It was also hypothesized that traumatic brain injury would be associated with early bone union and increased callus formation. Furthermore, it was expected that the proliferation of osteoprogenitor cells in response to sera from patients with a traumatic brain injury would be positively related to the quantity of callus formed and the time to union of long-bone fractures. Additionally, this study investigated whether the extent of the central nervous system lesion was related to the degree of enhanced callus formation, shortened time to union, and the osteogenic potential of serum from patients with a traumatic brain injury.

## Materials and Methods

### Patients

Forty-one consecutive patients (thirty-one male and ten female patients between seventeen and seventy-two years of age) were recruited from Royal Perth Hospital, Perth, Western Australia, between January 2005 and February 2007 (Table I). Seventeen patients with a severe brain injury presenting with a long-bone fracture (femur, tibia, or humerus) were treated at our level-I trauma center according to the Advanced Trauma Life Support guidelines<sup>10</sup>. After primary diagnostic and therapeutic management, they were transferred to the intensive care unit. Twenty-four patients with an isolated shaft fracture of the femur, tibia, or humerus without a traumatic brain injury were recruited as control subjects. The overall mean age of the patients in the study was 32.4 years (range, seventeen to seventy-two years) and did not differ between treatment groups ( $p > 0.05$ ).

On admission, all patients were evaluated with use of the Glasgow Coma Scale<sup>11</sup> and Abbreviated Injury Scale score<sup>12</sup>. The type and extent of the hemorrhagic central nervous system lesion or diffuse cerebral edema were determined by computerized axial tomography and were scored with the Marshall computed tomography classification system<sup>13</sup>. Eligibility criteria for recruitment were met if the patient had a severe traumatic brain injury with a Glasgow Coma Scale score of  $\leq 8$  points and an Abbreviated Injury Scale score of  $> 2$ . All patients in the traumatic brain injury and fracture group sustained a severe traumatic brain injury and had a mean Glasgow Coma Scale score of 5.6 (range, 3 to 8) and a mean Abbreviated Injury Scale score of 3.9 (range, 2 to 5). The radiographic findings included

epidural, subdural, subarachnoid, and intraventricular hemorrhage as well as generalized brain edema and shearing injuries. The mean Marshall computed tomography classification for the patients with a traumatic brain injury was 2.9 (range, 2 to 4). In addition to the intracranial lesions, eight of the seventeen patients with a traumatic brain injury had sustained a skull fracture and nine required operative neurosurgical intervention. Clinical and radiographic investigation revealed that none of the control patients sustained a traumatic brain injury, and the mild deficits on the Glasgow Coma Scale were due to extracranial causes.

All patients sustained an isolated severe shaft fracture of the femur (twenty-eight patients), tibia (ten patients), or humerus (three patients). The long-bone fractures were imaged by conventional radiographs in anteroposterior and lateral projections. The fractures in all patients were treated operatively. All femoral fractures (fourteen patients in each group) were stabilized with an intramedullary nail with use of a reamed technique. The two groups were not significantly different in terms of the time interval to surgical treatment of the bone fracture (29.6 compared with 42.3 hours;  $p = 0.69$ ; Table I).

Exclusion criteria included all forms of prior nervous system or bone-related diseases, immunosuppression, rheumatoid arthritis, and diabetes as well as steroid or bisphosphonate therapy. No further selection was performed after study inclusion; only patients with at least a one-year survival were considered. Ethics approval was granted by the Royal Perth Hospital Human Research Ethics Committee, and all patients or next of kin gave informed consent.

### Specimen Collection

Blood samples were collected from all patients at four time points during the first week following the injury. The first sample was obtained as soon as possible after hospital admission, but always within six hours after the injury. Subsequent samples were collected at twenty-four, seventy-two, and 168 hours after the injury. All specimens (except the first sample after the injury) were obtained at approximately 0800 hours in order to reduce the circadian influence on biochemical markers and to maintain similar conditions for all participant groups. Samples were centrifuged at 1500 g for ten minutes within thirty minutes of collection, and the resulting serum was stored at  $-80^{\circ}\text{C}$ . The levels of C-reactive protein, alkaline phosphatase, adjusted calcium, inorganic phosphate, and parathyroid hormone were measured in all collected samples.

### Cells

The conditionally immortalized human fetal osteoblastic cell line hFOB1.19 was obtained from the American Type Culture Collection (ATCC, Manassas, Virginia). The cells were cultured according to ATCC protocols in a D-MEM/F-12 medium (DMEM [Dulbecco modified Eagle medium] Gibco; Invitrogen, Auckland, New Zealand) supplemented with 15% (volume per volume) fetal calf serum (FCS; JRH Biosciences, Lenexa, Kansas) and 1% antibiotics (10,000 U/mL of penicillin-G sodium, 10,000  $\mu\text{g}/\text{mL}$  of streptomycin sulfate, and 25  $\mu\text{g}/\text{mL}$  of amphotericin B;

**TABLE I Demographic and Clinical Profile of Patients with Traumatic Brain Injury and Fracture and of Control Patients**

	Patients with Traumatic Brain Injury and Fracture	Patients with Fracture
No.	17	24
Gender (M/F)	10/7	21/3
Age* (yr)	28.4 ± 10.4 (18-58)	36.4 ± 14.2 (17-72)
Glasgow Coma Scale score*	5.6 ± 2.2 (3-8)†	14.8 ± 0.2 (14-15)
Abbreviated Injury Scale score*	3.9 ± 0.9 (2-5)	-
Fracture (femur/tibia/humerus)	14/1/2	14/9/1
Femoral implant (intramedullary nail)	14	14
Time to surgery‡ (hr)	29.6 (9-37)	42.3 (11-57)
Length of stay* (days)	106.8 ± 101.8 (21-384)†	24.2 ± 24.7 (3-74)

\*The values are given as the mean and the standard deviation, with the range in parentheses. †The difference between the groups was significant ( $p < 0.05$ ). ‡The values are given as the mean, with the range in parentheses.

Gibco) at 34°C under humidified atmosphere of 95% air and 5% carbon dioxide. The cells were used four days after passaging and before reaching confluence for the assays mentioned below.

#### Cell Proliferation Assay

The osteoinductive potential of the sera was quantified by measuring the proliferation of the osteoblastic cell line hFOB1.19<sup>8,9</sup>.

Briefly,  $10^4$  cells were seeded in ninety-six-well plates (Sarstedt, Nümbrecht, Germany) in 200  $\mu$ L of DMEM (containing 1% antibiotics) per well. Patient serum was added at a concentration of 6.25% and then was serially diluted to a final concentration of 0.78% in triplicates. Cell proliferation was assessed with use of the 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) colorimetric assay

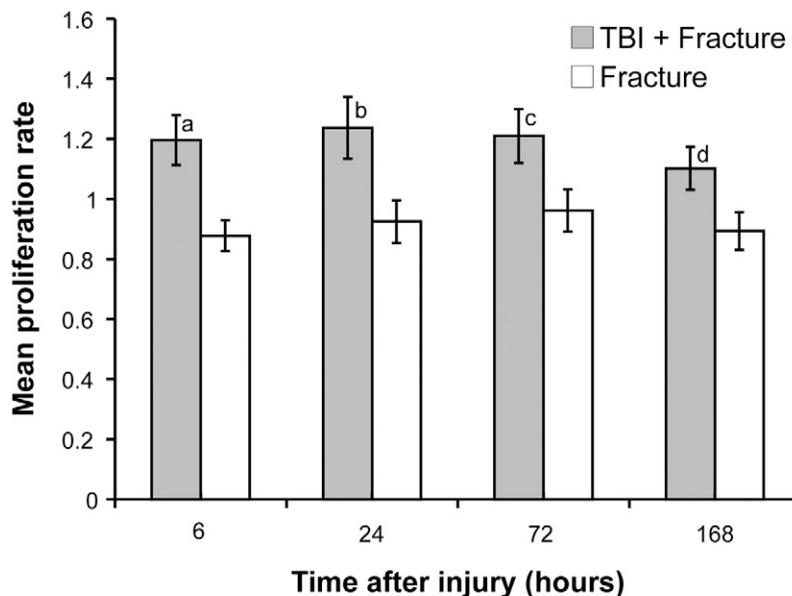


Fig. 1

The mean proliferation rates (and standard error) of hFOB cells measured after incubation with serum from seventeen patients with a traumatic brain injury (TBI) and twenty-four patients without a traumatic brain injury at six, twenty-four, seventy-two, and 168 hours after injury. The letters a and b indicate a significant difference between the groups ( $p = 0.02$ ), and the letters c and d indicate a significant difference between the groups ( $p = 0.04$ ).

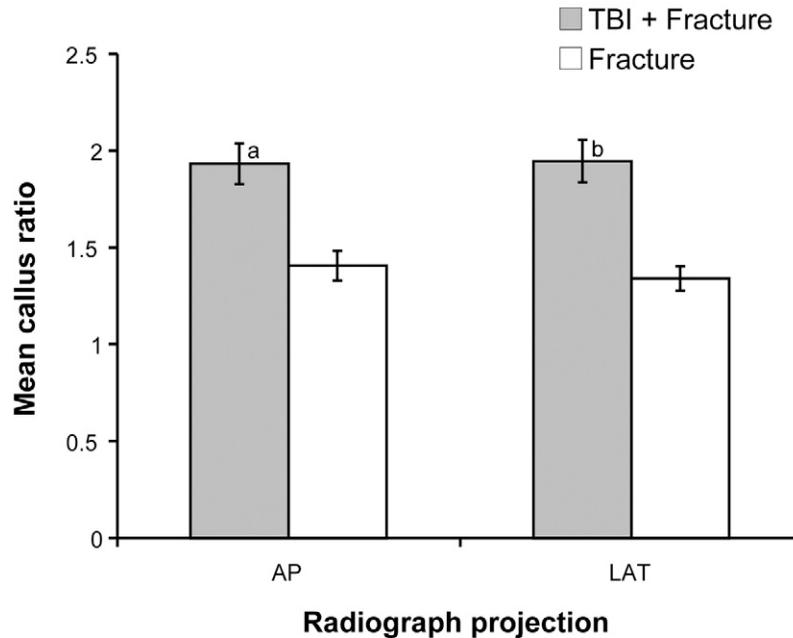


Fig. 2

The mean callus ratio (and standard error) of the maximal callus to diaphysis diameter adjacent to the fracture site on anteroposterior (AP) and lateral (LAT) radiographs. The letters a and b indicate a significant difference between the groups ( $p < 0.01$ ). TBI = traumatic brain injury.

(Promega, Madison, Wisconsin) and was recorded with use of a microplate reader (Multiskan RC; Labsystems, Helsinki, Finland) at an absorbance of 492 nm. The proliferation rates were calculated as the percentage of the negative control, which was the proliferation rate of the hFOB cells incubated with standard medium containing antibiotics and 0% fetal calf serum.

#### Reverse Transcription of mRNA and Real-Time Polymerase Chain Reaction Analysis

Messenger RNA was extracted from hFOB cells with use of ULTRASPEC RNA (Biotecx Laboratories, Houston, Texas) after four days of incubation, and mRNA was reverse-transcribed to cDNA with use of SuperScript III (Invitrogen). One-tenth of cDNA was amplified with use of a Platinum PCR SuperMix (Invitrogen) with gene-specific primers for osteoblast differentiation markers, including Sp7 (Osterix), alkaline phosphatase, and macrophage colony-stimulating factor. The amplified products were subjected to electrophoresis in 2% agarose gel and were stained with ethidium bromide (Sigma-Aldrich, St. Louis, Missouri). Primers used for the amplification are listed in a table in the Appendix.

#### Clinical and Radiographic Follow-up

All patients were followed clinically for a minimum of twelve months after discharge. Only patients with a closed diaphyseal femoral fracture were considered for the clinical and radiographic assessment of fracture-healing. Fourteen patients with a traumatic brain injury were compared with fourteen control patients. The patients were assessed at six weeks and at three,

six, and twelve months after injury. Clinical assessment of fracture-healing and heterotopic ossification (defined as ectopic bone in muscle and connective tissue surrounding joints without connection to the fracture callus) was investigated, and standard anteroposterior and lateral radiographs were made at all follow-up visits.

Two radiologists, blinded to the presence or absence of traumatic brain injury, independently evaluated time to union, defined as the time interval from treatment to bone-bridging of both cortices on the two radiographs. Callus formation and callus size were quantified with use of the method previously described by Spencer<sup>14</sup>. Briefly, the ratio was calculated with use of the widest callus diameter (measured at 90° to the long axis of the bone) and the diameter of the normal adjacent diaphysis on the anteroposterior and lateral radiographs. The highest ratio calculated during fracture-healing was used for further analysis.

#### Statistical Analysis

A two-way repeated-measures analysis of variance was used to test mean differences between the study groups in terms of proliferation rates of hFOB cells across the various time points after injury and in terms of serum markers. Independent-samples t tests were used to determine specific differences according to group and time point after injury. Pearson correlations (bivariate) were used to analyze two continuous variables, including patient age, proliferation rates, mean callus ratios, time to union, length of stay, Glasgow Coma Scale score, Abbreviated Injury Scale score, and serum marker concentrations. A p value of  $<0.05$  was considered significant. Retrospective

**TABLE II Correlation Between Mean Callus Ratios on Radiographic Projections and Proliferation Rates, Time to Union, and Glasgow Coma Scale Score for All Patients**

	Callus Ratio ( <i>r</i> value)	
	Anteroposterior Radiograph	Lateral Radiograph
Proliferation rate		
6 hr	0.49*	0.50*
24 hr	0.50*	0.50*
72 hr	0.40	0.47
168 hr	0.51*	0.49*
Time to union	-0.45*	-0.48*
Glasgow Coma Scale score	-0.55*	-0.58*

\*The correlation was significant (two-tailed Pearson correlation coefficient,  $p < 0.05$ ).

sample size calculations indicated that there were sufficient patient numbers for statistical comparisons between treatment variables.

#### Source of Funding

The funding was used to purchase reagents for this study.

#### Results

##### Cell Proliferation Assay and Real-Time Polymerase Chain Reaction Analysis

The patients with a traumatic brain injury had a higher mean proliferation rate of hFOB cells at all time points of sampling than did control patients (120% and 88%, respectively, of the internal negative control at six hours [ $p = 0.02$ ], 123% and 93% at twenty-four hours [ $p = 0.02$ ], 121% and 96% at seventy-two hours [ $p = 0.04$ ], and 110% and 89% at 168 hours [ $p = 0.04$ ]; Fig. 1). The sera of patients with a traumatic brain injury induced a greater expression of mRNA osteoblast differentiation markers (including Sp7 [Osterix], alkaline phosphatase, and macrophage colony-stimulating factor) than did that of the control patients (data not shown).

#### Radiographic Follow-up

The time to union of femoral fractures was shorter in the traumatic brain injury and fracture group than in the control patients (8.7 and 16.3 weeks, respectively;  $p = 0.01$ ). The mean callus ratio of the femoral fractures was considerably greater on both the anteroposterior and the lateral radiographs for the patients with a traumatic brain injury than for the controls (1.9 and 1.3, respectively, on the anteroposterior radiograph and 1.9 and 1.4 on the lateral projection [ $p < 0.01$ ]; Fig. 2). Radiographic evidence of heterotopic ossification was found in four of the seventeen patients with a traumatic brain injury and in one control. Sex or association with a skull fracture did not influence the mean callus ratio.

The proliferation rate of hFOB cells incubated with serum was positively correlated to callus ratios of the femur on both the anteroposterior and lateral radiographic projections at six, twenty-four, and 168 hours after the injury ( $p < 0.05$ ). Also of significance was an inverse linear relationship between the time to union and the mean callus ratio on both radiographic projections ( $p < 0.05$ ; Table II).

There was a negative linear relationship between the Glasgow Coma Scale score and the mean callus ratio on the anteroposterior and lateral projections for all patients ( $p < 0.05$ ; Table II and Appendix). The time to union was correlated to the Glasgow Coma Scale score for all patients ( $p = 0.04$ ; see Appendix). Additionally, a negative correlation was observed between the Glasgow Coma Scale score and the cell proliferation rate at six hours after injury ( $p = 0.03$ ; see Appendix). Analysis revealed a trend between the Abbreviated Injury Scale score for head injury and the mean callus ratio on both radiographic projections as well as between the Abbreviated Injury Scale score for head injury and the cell proliferation rates at six and twenty-four hours after injury.

#### Biochemical Markers

Plasma C-reactive protein levels were above the normal range at all time points for both the patients with a traumatic brain injury and the control patients (Table III). The C-reactive protein levels at 168 hours were higher in patients with a traumatic brain injury compared with control patients ( $p < 0.05$ ). There was a trend toward increasing serum alkaline phosphatase and decreasing parathyroid hormone concentrations over time in both

**TABLE III Mean Serum Marker Levels in Patients with and without Traumatic Brain Injury**

	Normal Range	Patients with Traumatic Brain Injury and Fracture*	Patients with a Fracture*
C-reactive protein level (mg/L)	<5	148.6 ± 42.3†	88.5 ± 15.9†
Alkaline phosphatase (U/L)	40-90	70.5 ± 4.8	77.0 ± 7.4
Calcium (mmol/L)	2.7-7.5	1.9 ± 0.1‡	2.2 ± 0.4‡
Phosphate (mmol/L)	0.84-1.45	1.1 ± 0.1	1.1 ± 0.3
Parathyroid hormone (pmol/L)	1.2-6.0	7.6 ± 1.0†	5.3 ± 0.6

\*The values are given as the mean and the standard deviation. †The values are above normal range. ‡The values are below normal range.

patient groups. There were no significant differences in alkaline phosphatase, phosphate, or parathyroid hormone concentrations between the groups at any time point, with the exception of parathyroid hormone at twenty-four hours, when patients with a traumatic brain injury had significantly higher parathyroid hormone levels compared with the control group (10.3 and 4.6 pmol/L, respectively;  $p < 0.05$ ). Serum calcium levels were significantly lower in the patients with a traumatic brain injury compared with control patients over all time points ( $p < 0.05$ ). There was an inverse correlation between hFOB proliferation rates and alkaline phosphatase levels at six hours after injury ( $p < 0.05$ ).

### Discussion

All hypotheses were supported by the results of this study. Patients with a traumatic brain injury and a femoral shaft fracture demonstrated enhanced fracture-healing with a two-fold shorter time to union ( $p = 0.01$ ) and a 37% to 50% increase in the mean callus ratio on both anteroposterior and lateral radiographic projections ( $p < 0.01$ ). These results are consistent with previous clinical studies that have described this phenomenon both in femoral<sup>1,15</sup> and other long-bone fractures<sup>16</sup>. To date, the exact pathophysiologic mechanism responsible for this phenomenon remains controversial. However, there is a consensus regarding enhanced fracture-healing in patients with associated traumatic brain injury.

The hypothesis that serum from patients with a traumatic brain injury would have a greater proliferative influence on the osteoprogenitor cell line hFOB1.19 than would samples from patients without a traumatic brain injury was supported. There has long been a debate as to whether a humoral factor is released from the injured brain, or whether a direct nervous action takes place for the induction of enhanced fracture-healing. Several studies have suggested that a combination of a signaling cascade involving humoral, neuronal, and local factors may play an important role after trauma<sup>17,18</sup>. In this study, we used the hFOB cell line to screen serum samples for the presence of osteoinductive factors on the basis of the assumption that these factor(s) would be released by the damaged brain tissue and would induce the proliferation of stromal stem cells and their differentiation toward the osteoblast pathway. The hFOB cells are stromal stem cells at an early stage of differentiation, but naturally directed toward osteoblasts, and we therefore expected any putative osteoinductive factors in the serum of patients with a traumatic brain injury to enhance proliferation of these cells *in vitro*<sup>19</sup>. We demonstrated increased proliferation rates at all time points after trauma in the patients with a traumatic brain injury compared with the control group. Furthermore, sera of patients with a traumatic brain injury induced a higher mRNA expression of selected osteoblast markers. Both of these results support the concept of a humorally released osteoinductive factor(s) by the injured tissue. Beside the positive feature of enhancing fracture-healing, the assumed osteoinductive factors are related to a harmful increased prevalence of heterotopic ossification. Heterotopic ossification is characterized by the formation of lamellar bone at ectopic sites including

muscles and the connective tissue surrounding joints<sup>20</sup>. It may result in clinical complications, such as complete ankylosis in 10% to 16% of patients<sup>21,22</sup>. The prevalence of heterotopic ossification after severe traumatic brain injury is between 11% and 25% and is consistent with our observation<sup>23</sup>.

The results of our cellular experiments are in line with previous studies with use of animal models. Bidner et al.<sup>5</sup> reported an osteoblast-specific mitogenic effect of serum from patients with a head injury and were the first, as far as we know, to postulate that a humoral mechanism may be implicated in the association of osteogenesis with traumatic brain injury. Boes et al.<sup>4</sup> provided evidence for a humorally mediated mitogenic effect on osteoprogenitor cells by showing that *in vitro* human stromal stem cells proliferated beyond expected parameters when incubated with serum from rats with a brain injury. Furthermore, Renfree et al.<sup>19</sup> incubated fetal rat osteoblasts with sera from patients with a traumatic brain injury and observed a substantial increase in mitogenic activity in all patients.

In our study, the osteoinductive potential of serum showed a maximum activity as early as six hours after injury, remaining at the same level for three days before declining one week after trauma. This is in line with the hypothesis that the osteoinductive effect could reflect the disruption of the blood-brain barrier because of the trauma, allowing leakage of osteoinductive cerebrospinal fluid components into the systemic circulation<sup>24</sup>. In addition to the disruption of the blood-brain barrier, newly synthesized factors by the traumatized brain in the context of early repair mechanisms could play a role under such conditions. The reduction of circulating activity after approximately one week might be due to a fall in the production of osteogenic factors by the central nervous system, an increased clearance, a regeneration of the blood-brain barrier, or a combination of these events. However, no conclusion can be drawn about the exact time point of factor release, and additional research is necessary to determine how long such a putative factor may remain present and active in the systemic circulation. Ongoing studies aim to identify the osteoinductive factor(s) at the molecular level. Identification of the osteoinductive factor(s) will provide a major contribution to the understanding of pathophysiologic processes involved in the interaction between the injured brain and fractured bone.

We demonstrated that callus formation is positively correlated with the mitogenic potential of serum from patients with a traumatic brain injury. We showed that the extent of brain injury is directly related to the mitogenic potential of serum in the first six hours after the injury, time to union, and the degree of enhanced callus formation at the fracture site. This suggests that one or more osteogenic factors may be released by the injured brain tissue as early as six hours after trauma to influence the extent of repair tissue formed at the fracture zone during the healing process. Furthermore, the amount of callus formed appears to be dependent on the mitogenic potential of serum. As such, it could be suggested that there is a dose-dependent relationship between the

amount of callus formed and the quantity of osteogenic factors released by the injured brain, which in turn may be due to the extent of the brain injury. Although the severity of brain injury as measured on computed tomography scans of patients with a traumatic brain injury did not correlate significantly with the osteogenic potential of serum, there was a positive trend between these variables. Furthermore, brain injury severity as well as the mitogenic potential of serum from patients with a traumatic brain injury appeared to be negatively related to time to union, supporting the hypothesis mentioned above; however, these variables themselves were not significantly related.

### Appendix

**eA** A table showing the specific primer sequences used in this study and graphs correlating the Glasgow Coma Scale score with callus ratio, time to union, and cell proliferation rates are available with the electronic versions of this article, on our web site at [jbjs.org](http://jbjs.org) (go to the article citation and click on "Supplementary Material") and on our quarterly CD/DVD (call

our subscription department, at 781-449-9780, to order the CD or DVD). ■

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### References

- Giannoudis PV, Mushtaq S, Harwood P, Kambhampati S, Dimoutsos M, Stavrou Z, Pape HC. Accelerated bone healing and excessive callus formation in patients with femoral fracture and head injury. *Injury*. 2006;37 Suppl 3:S18-24. Erratum in: *Injury*. 2007;38:1224.
- Perkins R, Skirving AP. Callus formation and the rate of healing of femoral fractures in patients with head injuries. *J Bone Joint Surg Br*. 1987;69:521-4.
- Newman RJ, Stone MH, Mukherjee SK. Accelerated fracture union in association with severe head injury. *Injury*. 1987;18:241-6.
- Boes M, Kain M, Kakar S, Nicholls F, Cullinane D, Gerstenfeld L, Einhorn TA, Tornetta P 3rd. Osteogenic effects of traumatic brain injury on experimental fracture-healing. *J Bone Joint Surg Am*. 2006;88:738-43. Erratum in: *J Bone Joint Surg Am*. 2006;88:1602.
- Bidner SM, Rubins IM, Desjardins JV, Zukor DJ, Goltzman D. Evidence for a humoral mechanism for enhanced osteogenesis after head injury. *J Bone Joint Surg Am*. 1990;72:1144-9.
- Waters KM, Rickard DJ, Riggs BL, Khosla S, Katzenellenbogen JA, Katzenellenbogen BS, Moore J, Spelsberg TC. Estrogen regulation of human osteoblast function is determined by the stage of differentiation and the estrogen receptor isoform. *J Cell Biochem*. 2001;83:448-62.
- Subramaniam M, Jalal SM, Rickard DJ, Harris SA, Bolander ME, Spelsberg TC. Further characterization of human fetal osteoblastic hFOB 1.19 and hFOB/ER alpha cells: bone formation in vivo and karyotype analysis using multicolor fluorescent in situ hybridization. *J Cell Biochem*. 2002;87:9-15.
- Gautschi OP, Joesbury K, Meagher J, Skirving A, Filgueira L, Zellweger R. Serum-mediated osteogenic effect of traumatic brain injured patients. *J Am Coll Surg*. 2006;203:S37.
- Gautschi OP, Toffoli AM, Joesbury KA, Skirving AP, Filgueira L, Zellweger R. Osteoinductive effect of cerebrospinal fluid from brain-injured patients. *J Neurotrauma*. 2007;24:154-62.
- Collicott PE, Hughes I. Training in advanced trauma life support. *JAMA*. 1980;243:1156-9.
- Teasdale G, Jennette B. Assessment of coma and impaired consciousness. A practical scale. *Lancet*. 1974;2:81-4.
- Abbreviated injury score (updated). Des Plaines, IL: Association for Advanced Automotive Medicine; 1998.
- Marshall LF, Marshall SB, Klauber MR, Van Berkum Clark M, Eisenberg H, Jane JA, Luerksen TG, Marmarou A, Foulkes MA. The diagnosis of head injury requires a classification based on computed axial tomography. *J Neurotrauma*. 1992;9 Suppl 1:S287-92.
- Spencer RF. The effect of head injury on fracture healing. A quantitative assessment. *J Bone Joint Surg Br*. 1987;69:525-8.
- Newman RJ, Stone MH, Mukherjee SK. Accelerated fracture union in association with severe head injury. *Injury*. 1987;18:241-6.
- Garland DE, Todler L. Fractures of the tibial diaphysis in adults with head injuries. *Clin Orthop Relat Res*. 1980;150:198-202.
- van Kuijk AA, Geurts AC, van Kuppevelt HJ. Neurogenic heterotopic ossification in spinal cord injury. *Spinal Cord*. 2002;40:313-26.
- Trentz OA, Handschin AE, Bestmann L, Hoerstrup SP, Trentz OL, Platz A. Influence of brain injury on early posttraumatic bone metabolism. *Crit Care Med*. 2005;33:399-406.
- Renfree KJ, Banovac K, Hornicek FJ, Leibold NH, Villanueva PA, Nedd KJ. Evaluation of serum osteoblast mitogenic activity in spinal cord and head injury patients with acute heterotopic ossification. *Spine*. 1994;19:740-6.
- Vanden Bossche L, Vanderstraeten G. Heterotopic ossification: a review. *J Rehabil Med*. 2005;37:129-36.
- Garland DE. A clinical perspective on common forms of acquired heterotopic ossification. *Clin Orthop Relat Res*. 1991;263:13-29.
- Wharton GW, Morgan TH. Ankylosis in the paralyzed patient. *J Bone Joint Surg Am*. 1970;52:105-12.
- Kaplan FS, Glaser DL, Hebel N, Shore EM. Heterotopic ossification. *J Am Acad Orthop Surg*. 2004;12:116-25.
- Klein BY, Shohami E, Reikhshtein Y, Ben-Bassat H, Liebergall M. Serum-mediated osteogenic effects of head injury on cultured rat marrow stromal cells. *Calcif Tissue Int*. 1999;65:217-22.