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Endothelial Progenitor Cells Correlate with Clinical Outcome of Traumatic Brain Injury

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Abstract

Objective—Endothelial progenitor cells (EPCs) play an active role in vascular repair and revascularization of tissue damaged by traumatic, inflammatory, and ischemic injuries. We correlate the changes in circulating EPCs with the severity of traumatic brain injury (TBI). The study is designed to investigate the EPC mobilization after injury and a potential use of circulating EPCs as a prognostic marker for evaluating trauma severity and clinical outcomes.

Design—A prospective cohort study conducted in two neurosurgical intensive care units (NSICU) of Tianjin Medical University General Hospital and Tianjin Huanhu Hospital.

Patients—Patients with traumatic brain injury and age- and gender-matched healthy controls.

Interventions—None

Measurements and Main Results—Changes in the levels of circulating EPCs were monitored for up to 21 days in 84 patients with TBI. Results were correlated with the clinical assessment of injury severity as determined by Glasgow Coma Scale (GCS). The level of circulating EPCs was found to be suppressed 24–48 hrs after injury, but rapidly increased, reaching the highest at day 5–7 posttrauma. Circulating EPCs in patients with improved GCS were significantly higher than those with deteriorated conditions, and remained persistently low in patients who died of trauma.

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Conclusions—The results suggest that the level of circulating EPCs correlates with the clinical severity and outcome of TBI, and may offer potentials as a prognostic marker for TBI. A long-term follow up of these patients is ongoing.

Keywords

Endothelial Progenitor Cells; Traumatic brain injury; Clinical outcome; Prognostic marker

Since their first identification more than a decade ago (1), endothelial progenitor cells (EPCs) have been extensively studied for their ability to repair damaged vessel walls and to form new vasculature (2). EPCs are the lineage-specific stem cells that are mobilized from the bone marrow to peripheral blood in response to traumatic, inflammatory, and ischemic injury (3–5). They are defined as a subpopulation of CD34+ hematopoietic cells that express CD133 and vascular endothelial growth factor receptor 2 (VEGFR-2) (6) and are capable of proliferation and differentiation into mature endothelial cells (6, 7). CD133 is predominantly expressed on “early” EPCs and lost overtime when cells begin to express von Willebrand factor, a multimeric adhesion molecule that is synthesized in mature endothelial cells and stored in the Weibel Palade bodies (6, 8, 9). It is because of this ability that EPCs have been increasingly recognized as a key cellular component for vascular repairs after injury (2, 9, 10).

Upon vascular injury, EPCs are mobilized from the bone marrow to blood circulation to eventually “home” to the site of injury. Platelets play an active role in the EPC homing and subsequent differentiation (11–13). Consistent with such a role, the levels of circulating EPCs rapidly increase in a variety of pathologic conditions; including diabetes, systemic lupus erythematosus, chronic renal failure, rheumatoid arthritis, cancer, coronary artery disease (including vascular stenting), and trauma (14). Systemic inflammation is the common characteristic shared among these rather diverse clinical conditions, indicating that stress may be a trigger for EPC mobilization. However, the clinical significance of circulating EPCs in traumatic brain injury (TBI), the acute and most dramatic stress, remains largely unknown.

Vascular repair in the TBI-induced brain damage may be more important than other organs because brain is most sensitive to ischemic injury occurring secondary to mechanical impacts. We have recently demonstrated that EPCs in peripheral blood undergo a bidirectional changes in patients with TBI in a smaller cohort of 29 patients (15). However, the number of patients recruited to the study is insufficient to conduct statistical analyses based on clinical course and prognosis. More importantly, the extent of traumatic injury varies among patients and the variation is further compounded with differential treatments, both of which could have significantly impact on EPC levels and reactivity in peripheral blood and at the site of vascular injury. To address these issues, we have expended our initial study to include more patients (101 were recruited and 84 finally analyzed). The larger sample size allowed us to conduct subgroup analysis to determine whether circulating EPC levels correlate with clinical TBI prognosis during a follow-up period of up to 21 days.

MATERIALS AND METHODS

Patients

Patients with TBI, who were admitted to Tianjin Medical University General Hospital and Tianjin Huanhu Hospital from April 2006 to March 2007, were enrolled. The exclusion criteria include: patients with complex trauma involving body trunk and limbs, hematologic disorders, cancer and on sedation. A total of 101 patients and 37 age- and gender matched healthy controls were recruited for the study. The study was approved by the Institutional Review Board of Tianjin Medical University for the use of human subjects in biomedical research. All subjects or guardians signed consent form before enrollment and blood samples were collected.

Morning fasting venous blood (2 mL) was collected at 1, 4, 7, 14, and 21 days after TBI using ethylenediaminetetraacetic acid (EDTA, 0.5 mM final concentration) as anti-coagulant. Blood samples were also collected from age and gender-matched healthy subjects to obtain baseline normal reference values, which was used to evaluate changes in circulating EPCs in patients with TBI. Blood samples were processed within 2 hr after blood draw.

EPC Isolation and Measurements

To quantify EPCs in circulation, peripheral mononuclear cells were first isolated from blood samples as previously described (15). Briefly, 2 mL of blood was subjected to a density gradient centrifugation at $300 \times g$ for 20 min at room temperature. The isolated cells were washed three times with phosphate-buffered saline (PBS; pH 7.2) and resuspended in 200 μ L of PBS supplemented with 0.5% of bovine serum albumin (BSA) and 2 mM of EDTA. The cells were then labeled with a PE (R-phycoerythrin)-conjugated monoclonal CD133 antibody (Miltenyi Biotech, Gladbach, Germany) or FITC (Fluorescein Isothiocyanate)-conjugated CD34 monoclonal antibody (BD Pharmingen, San Jose, CA) or both for 20 min at room temperature. We used two cellular markers to identify peripheral EPCs because CD34 recognizes stem cells of endothelial lineage, whereas CD133 primarily detects immature EPCs that are highly capable of differentiating into endothelial cells. The double staining will allow us to not only identify endothelial cells in circulation, but also determine the ratio of mature to immature cell populations, a measure that can potentially be used to gauge patient's ability to repair the vasculature. Stained cells were washed with PBS/BSA and then analyzed by flow cytometry (BD FACS Calibur, BD Biosciences, San Jose, CA). Two isotype controls of PE- and FITC-conjugated mouse immunoglobulin G (IgG) were used for background nonspecific binding. Cells were first run on forward and side scatter to select mononuclear cells to reduce signal noises from cell aggregates, platelets and cellular debris. Three populations of cells were analyzed: CD34₊CD133₊ and double-stained cells, and expressed as numbers of positive cells in each population. For double fluorescence detection, cells were first gated for their CD34 positivity and then for CD133 staining. EPCs were then detected as gated cells that were stained for FITC-CD34 and PE-CD133 and quantified as the number of EPCs per 2×10^6 mononuclear cells (15).

Clinical Assessment of Neurologic Recovery

All patients admitted were treated according to the international Guidelines for TBI treatment. To our best knowledge, patients did not use medicines that are known to affect the level of circulating EPCs during the follow-up period. All patients were diagnosed based on CT or MRI scans that showed the evidence of intracranial hematoma, hemorrhage, and cerebral contusion. Patients were scored upon admission by Glasgow Coma Scale (GCS) and divided into mild (GCS 13–15) and moderate-severe (GCS $\leq$ 12) injury. The GCS score of survival patients was also monitored during the follow up period to determine whether a patient had 1) improved (unchanged for those with GCS of 15 or showed improvement of ≥ 1 point); 2) deteriorated (GCS remained unchanged or lowered ≥ 1 point), or 3) death.

Statistical Analysis

All statistical analyses were performed by a biostatistician who was blinded about demographic information, clinical diagnosis, outcomes and EPC measurements of individual patients. The data are expressed as mean $\pm$ SEM for continuous variables and as percentage for categorical variables. Categorical variables were compared using the Pearson chi-square test. Comparisons between groups for continuous variables were made using the Student's *t* test or one-way ANOVA followed by SNK test. Univariate correlations were performed using Pearson correlation coefficients (for continuous values) or Spearman correlation coefficients (for categorical values).

Receiver operator characteristics (ROC) curves and the areas under the curves (AUCs) for increase in circulating EPCs were analyzed and used to predict neurologic recovery or short-term outcome as measured by the improvement of GCS score. Repeated measures ANOVA with posthoc LSD test was used to compare the number of circulating EPCs at different time points between patients with improved and those with deteriorated outcomes. The analysis of mixed-effects models was used to assess the dynamic changes of circulating EPCs numbers for patients who survived and those who did not during the follow-up period.

The influence of the circulating EPCs elevation in the first 7 days on neurologic recovery or short-term outcome was assessed by logistic regression analysis. Results were expressed as adjusted odds ratios with the corresponding 95% CI. A *p* value of <0.05 was considered to be statistically significant. All analyses were performed using the SPSS software (Version 16.0; SPSS Inc, Chicago) or a SAS statistical package (Version 8.1; SAS Institute, Cary, NC).

RESULTS

Patient

A total of 101 patients were initially recruited, but 84 patients were included in final statistical analysis. Seventeen patients were excluded due to a large amount of blood transfusion, which interfered with the levels of patient-derived EPCs (data not shown). To address the concern of blood transfusion, we have conducted a pilot study and determined that the levels of circulating EPC in patients who received <1800 mL of blood transfusion (mean 856 mL) were not statistically different from those without blood transfusion during

the follow-up period (Fig. 1A). Based on this pilot study, patients who received >1800 mL of blood transfusion were excluded from the final analyses. The demographic information of the patients and controls was presented in Table 1. Among 84 patients included in data analyses, 57 underwent open surgery to remove hematoma and/or to relieve intracranial hypertension and 27 received nonsurgical treatments. There was no significant difference between subjects underwent craniotomy and those without surgery (Fig. 1B). As a result, we exclude surgery and blood transfusion of <1800 mL as confounding factors for data stratification.

Of the 84 patients analyzed, 69 patients survived and 15 died during the follow up period. Among survival patients, the clinical conditions were improved in 62 patients and deteriorated in 7 patients (in some analyses, death and deterioration were combined due to a limited number of cases). To determine the prognostic value of EPCs in peripheral blood samples, patients were divided into two categories of data analyses. First was to show differences between survivors and nonsurvivors (Analysis 1) and second to show differences between improving and nonimproving (including death) patients (Analysis 2) during the course of a follow up period of 21 days.

Clinical variables of patients in the two categories were listed in Table 2. GCS measured upon admission and during follow up was significantly lower for nonsurvival patients and patients with deteriorated conditions as compared to survival patients and those with improved clinical conditions. Blood glucose levels followed a similar trend, showing that patients who were died of TBI and those with deteriorated conditions during the follow up period had significantly higher blood glucose than survival patients and patients with improved clinical conditions.

Circulating EPC in Patients and Controls

The average level of circulating EPCs for all patients was significantly lower in the first 24 hr after TBI as compared to healthy subjects. However, it was rapidly increased thereafter, reaching the plateau 7 days post TBI and then gradually declining to the baseline level over the course of 2 wks (Fig. 2).

The changes in circulating EPCs from day 1 to 7 were shown in Figure 3A, showing a typical biphasic distribution in survival patients. However, this biphasic distribution was not found in patients who were died of TBI. The number of circulating EPCs was significantly lower in patients died of TBI as compared to those who survived (41.78 ± 12.70 vs. 71.53 ± 9.17 , $p < .01$). A mixed-effected model described the dynamic changes of circulating EPC during follow-up detected statistically different intercepts ($p = .034$) and slopes ($p = .0015$) between survival and nonsurvival patients.

For survival patients, the numbers of circulating EPCs on day 7, 14 and 21 were significantly higher for patients with improved conditions as compared to those with deteriorated conditions ($p < .01$, Fig. 3B). More importantly, the magnitude of increase in circulating EPCs was markedly greater for patients with improved conditions as compared those with deteriorated conditions, who actually showed a steadily decline in EPC levels during the follow up period (Fig. 3C). EPC counts in peripheral blood showed a steady

increase over time for most patients with improved clinical conditions (Fig. 4A), whereas they remained unchanged for those with deteriorated conditions during the same period (Fig. 4B). Due to a lack of linearity, the number of circulating EPCs in the first 7 days was also analyzed by receiver operating characteristic curves and areas under the curve (AUC, Fig. 4C). The analysis identified 41 EPCs/ 10^6 mononuclear cells of blood sample as the cut-off point for a sensitive and specific outcome prediction (Fig. 4, AUC = 0.691; 95% CI = 0.581 to 0.802, $p = .008$). The logistic regression analysis (Table 3) showed that the number of circulating EPCs equal or >41 per 10^6 mononuclear cells in the first 7 days independently predicts neurologic recovery (odds ratio: 0.988; 95% CI, 0.977 to 0.999; $p = .029$) after adjusted for GCS, body temperature, blood glucose upon admission and type of injury. More importantly, there is a significant positive correlation between circulating EPC numbers on day 7 and neurologic recovery (Fig. 5, survivor vs. nonsurvivor, $R_2 = 0.1298$, $p < .001$ and improved vs. deteriorated, $R_2 = 0.0893$, $p < .001$).

Furthermore, a Kaplan-Meier survival estimate based on the magnitude of increase in circulating EPCs in the first 7 days finds that there is a higher hospital mortality for patients, whose circulating ECP level is equal or higher than 41 EPCs per 10^6 mononuclear cells in the first 7 days (Fig. 6).

DISCUSSION

Since the first report by Asahara M, et al in 1997 (1), endothelial progenitor cells isolated from peripheral blood have been the focus on extensive studies to characterize their phenotype, biochemical properties, and more importantly, to evaluate as a potential therapeutic and diagnostic tool. Here, we presented data from a clinical study to specifically examine the dynamic change of EPC counts in peripheral blood and its prognostic value for traumatic brain injury. Consistent with our early report of a smaller cohort of patients (15), EPCs in peripheral blood were first suppressed to the level that is 72.6% of normal reference within the first 24–48 hrs of trauma and then increased steadily, reaching a peak level of 173.1% of normal reference at approximately 7 days before declining to baseline (Fig. 2). The initial EPC suppression is also reported in patients with ischemic stroke (4) and is likely to be consumptive in nature as circulating EPCs accumulate at the injured vascular beds. The notion is supported by our recent observation that CD34 $^{+}$ hematopoietic stem cells (EPC is a subpopulation of CD34 $^{+}$ cells) accumulate in the injured cerebral tissue and are found to be incorporated into new vasculature in TBI rats (16). The subsequent increase in EPC levels is likely resulted from the bone marrow mobilization in response to TBI as well as the consumptive EPC deficiency. One of the key findings of the current study is that the increase in circulating EPCs is associated with an improved clinical outcome in TBI patients (Fig. 3D & Fig. 5).

This association demonstrates the importance of circulating EPCs in vascular repairs. Traumatic injury can cause brain damage by direct mechanical impact, but more importantly by ischemic injury secondary to intracranial hypertension, tissue hypoxia and microthrombosis. The damaged cells could subsequently release active substances such as pro-inflammatory cytokines to propagate tissue injury. It is therefore critical to reestablish blood circulation early. There is abundant evidence to suggest that peripheral EPCs actively

participate in tissue repair, largely through vascular repair and angiogenesis to restore blood flow to the injured area. Circulating EPCs have been found to rapidly accumulate in the ischemic area to form new vessels and improve regional blood flow (17). The number of circulating EPCs is reversely correlated with the size of ischemic area (4). Most clinical observations indicate that the secondary injury occurs approximately 3–5 days after trauma, when tissue repair is also most active (18). The most significant neural improvements are commonly achieved by the third week. This course of vascular and neural repairs coincides with the pattern of changes in circulating EPC counts (Fig. 3C), with the highest EPC levels found at approximately 7 days after trauma.

A critical question is whether any of these change in circulating EPCs correlates with clinical course, treatment, and prognosis of patients. Our data suggest a strong correlation between circulating EPCs and clinical outcomes. Patients with worsened clinical outcome or death had a persistent low level of EPCs with limited or no increase observed for patients who had improved outcomes (Fig. 3 & 5), suggesting that EPCs may not have been sufficiently mobilized. Factors that limit or prevent EPC mobilization remain to be identified, but we find that the lack of insufficient EPC mobilization is not related to any particular type of injury or treatment. Consistent with the conclusion, the number of circulating EPCs has previously been demonstrated to correlate with the Framingham risk score for ischemic stroke (19). Yip et al (20) also shows that patients with severe neurologic dysfunction (NIHS score $\pm$ 12) have lower peripheral EPCs 48 hrs post ischemic stroke.

In summary, we have demonstrated a close correlation between the levels of circulating EPCs and short-term clinical outcomes of 84 patients with TBI. The EPC levels in peripheral blood were found to be first suppressed in the acute phase (first 24–48 hrs) and then rapidly increased, reaching the maximal level 7 days after trauma. Patients with consistent low circulating EPCs associated with worsened clinical conditions or death. Furthermore, the level of circulating EPCs was not affected by the type of injury and treatments. These results suggest that circulating EPCs may serve as a clinical prognostic marker for TBI. The current study is limited to correlating EPCs to short-term outcomes, but can be further expended into a multicentered study to develop a better regression model and to evaluate the quality of life for surviving patients in a longer follow up period. They also demonstrate that EPC transplantation or therapeutic means to increase EPC mobilization may improve patient recovery from severe traumatic brain injury.

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Figure 1. Effect of blood transfusion and craniotomy on the levels of circulating EPCs

A the peripheral EPC counts in patients who received <1800 mL of blood transfusion were compared to those without blood transfusion. The data were examined with repeated measure ANOVA showing not statistical different between the two groups of patients (transfusion: $F = 2.012$, $p = .160$; time course: $F = 7.659$, $p = .000$; the transfusion and time course interaction: $F = 1.485$, $p = .206$; the P values shown on tops of the bar graphs). **B** A comparison was also made between patients underwent open surgery and those without surgical intervention using repeated measure ANOVA (transfusion: $F = 3.124$, $p = .112$; time course: $F = 8.734$, $p = .000$; transfusion and time course interaction: $F = 1.713$, $p = .186$; the P values were listed on tops of the bar graphs).

Figure 2. Changes of circulating EPCs after TBI

EPC counts in peripheral blood samples were measured at five time points during a follow up period of up to 21 days. Blood samples were also collected from 37 healthy subjects as controls to set the normal reference value for baseline levels of circulating EPCs (the Student's *t* test, * $p < .01$).

Figure 3. Circulating EPCs and neurologic recovery of patients with TBI

A A comparison of dynamic changes in peripheral EPC counts between survival and nonsurvival patients in the first 7 days after TBI (paired *t* test, * $p < .01$). **B** Similarly, the number of circulating EPCs were also compared between patients with improved and those with deteriorated clinical conditions during the follow up period of 21 days (paired *t* test, *p* values are indicated on each group of bar graphs). **C** The magnitude of changes in peripheral EPC counts during the follow up period was also measured to more accurately monitor the dynamic changes in EPC counts during follow up (paired *t* test, *p* values were indicated on bar graphs).

Figure 4. The magnitude of elevation in circulating EPC and early neurologic recovery
 The actual tracing of EPC counts in peripheral blood samples collected at multiple time points during follow up period for patients with improved **A** and deteriorated **B** clinical conditions. A model of receiver operator characteristic curves (ROC) was performed. The elevation of circulating EPCs to equal or great than 41 per 10^6 mononuclear cells is calculated to be the cut-off point as the end point to sensitively and specifically predict clinical outcomes (AUC [area under the curve] = 0.691, 95%CI = 0.581 to 0.802, $p = .008$).

Figure 5. Relationship between the numbers of circulating EPCs on day 7 and neurologic recovery

There was a significant positive correlation between EPC counts in peripheral blood samples and neurologic recovery when a comparison was made between survival and nonsurvival patients **A** Analysis one, $R_2 = 0.1298$, $p < .001$) and between patients with improved clinical outcome and those with deteriorated conditions **B** Analysis two, $R_2 = 0.1298$, $p < .001$).

Figure 6. Relationship between circulating EPCs and early hospital mortality

The Kaplan-Meier estimate of survival according to the magnitude of elevated levels of peripheral EPC counts in the first 7 days was performed. Although most patients showed increase in EPC counts during the first 7 days, there was a higher rate of hospital mortality for patients, whose peripheral ECP counts increased, but remained below 41 per 10^6 mononuclear cells as compared to those with higher EPC counts measured during the first 7 days after TBI.

Figure 7.

Table 1

Demographic information of study cohort

	Healthy subjects (n=37)	Patients with TBI (n=84)	P*
Age, yrs			
Mean $\pm$ SEM (Range)	42.92 $\pm$ 2.67 (24–72)	40.45 $\pm$ 1.97 (18–85)	0.478
Sex, n (%)			
Female	9(24.3)	21 (25)	0.937
Pre-medical history, n (%)			
Hypertension	3 (8.10)	6(7.14)	0.852
Diabetes mellitus	1(2.70)	2(2.38)	0.916
Hyperlipidemia	4(10.81)	10(11.90)	0.862
Ischemic heart disease	1(2.70)	1(1.19)	0.548
stroke	2(5.40)	3(3.57)	0.641
Smoking habits, n (%)	4(10.81)	6(7.14)	0.500
Alcohol consumption, n (%)	1(2.70)	2(2.38)	0.916
Medication, n(%)			
Statins	3(8.11)	6(7.14)	0.852
Aspirin	4(10.81)	9(10.71)	0.987
Estrogen	1(2.70)	0	0.130

Statistical significances of difference between groups were determined by the dependent t test (continuous values) or by using the chi-square test (categorical values). TBI=traumatic brain injury.

Table 2

Baseline clinic characteristics of patients in two categories

	Analysis 1		P	Analysis 2		P
	Survivors(n=69)	Non-survivors (n=15)		Improvement (n=62)	Deteriorate (n=22)	
Age, yrs						
Mean $\pm$ SEM	39.55 $\pm$ 2.10	44.60 $\pm$ 5.41	0.330	38.48 $\pm$ 2.15	46.00 $\pm$ 4.35	0.094
(Range)	(18–85)	(18–75)		(18–85)	(18–75)	
Sex, n (%)						
Female	19(27.54)	2(13.33)	0.250	18(29.03)	3(13.64)	0.179
Time from injury to admission, (median, h)	6.00	3.00	0.314	6.00	3.00	0.264
Biochemistry and vital signs on admission						
GCS on admission*—median	11	4	0.000	12	4	0.000
Body temperature (median, °C)	37.20	37.21	0.290	37.24	37.31	0.689
Glucose levels on admission* (mean $\pm$ SE, mg/dl)	127.93 $\pm$ 7.06	186.03 $\pm$ 30.51	0.007	129.22 $\pm$ 7.29	171.52 $\pm$ 27.15	0.036
Mechanism of injury, n(%)						
Motor vehicle	31(44.93)	8(53.33)		28(45.16)	11(50.00)	
Fall	11(15.94)	1(6.67)	0.520	10(16.13)	7(31.82)	0.136
Assault	22(31.88)	6(40.00)		20(32.26)	2(9.09)	
Other	5(7.25)	0		4(6.45)	2(9.09)	
CT finding, n (%)						
Extradural hematoma	22(31.88)	8(53.33)	0.116	21(33.87)	4(18.18)	0.772
Subdural hematoma	19(27.54)	2(13.33)	0.250	17(27.42)	7(33.33)	0.188
Cerebral edema	13(18.84)	6(40.00)	0.076	12(19.35)	8(38.10)	0.083
Subarachnoid Hemorrhage(SAH)	32(46.37)	9(28.13)	0.339	30(48.39)	11(52.38)	0.752
Skull fracture	34(49.28)	6(40.00)	0.514	32(51.61)	11(36.36)	0.625
Types of Injury, n						
Open	13(18.84)	3(20.00)	0.917	50(80.65)	18(81.82)	0.904
Close	56(81.16)	12(80.00)		12(19.35)	4(18.18)	
Injury severity, n						
Mild	32(46.38)	1(6.67)		30(48.39)	3(13.64)	

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	Analysis 1		Analysis 2		P
	Survivors(n=69)	Non-survivors (n=15)	Improvement (n=62)	Deteriorate (n=22)	
Mediate	17(24.64)	3(20.00)	15(24.19)	5(22.73)	0.005
Severe *	20(28.98)	11(73.33)	17(27.42)	14(63.64)	
Surgical procedure, n (%)	44(63.77)	13(86.67)	39(46.43)	18(81.82)	0.103

Statistical significances of difference between analysis were determined by the Student's t test (continuous values) or by using the chi-square test (categorical values).

* P<0.05; TBI=traumatic brain injury.

Table 3

The effect of the elevation of circulating EPC numbers in the first 7 days on neural recovery during hospital days

	Crude		Adjusted	
	OR (95%CI)	<i>P</i>	OR (95%CI)	<i>P</i>
Elevation of circulating EPC numbers 41 in the first 7 days	0.989(0.981–0.997)	0.009	0.988(0.977–0.999)	0.029
GCS on admission			0.690(0.567–0.843)	0.000
Body temperature on admission			0.975(0.417–2.195)	0.917
Serum glucose in the first day			1.015(1.000–1.030)	0.044
Type of injury			0.719(0.678–0.901)	0.000