Original Investigation | CARING FOR THE CRITICALLY ILL PATIENT

Effect of Erythropoietin and Transfusion Threshold on Neurological Recovery After Traumatic Brain Injury
A Randomized Clinical Trial

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IMPORTANCE There is limited information about the effect of erythropoietin or a high hemoglobin transfusion threshold after a traumatic brain injury.

OBJECTIVE To compare the effects of erythropoietin and 2 hemoglobin transfusion thresholds (7 and 10 g/dL) on neurological recovery after traumatic brain injury.

DESIGN, SETTING, AND PARTICIPANTS Randomized clinical trial of 200 patients (erythropoietin, n = 102; placebo, n = 98) with closed head injury who were unable to follow commands and were enrolled within 6 hours of injury at neurosurgical intensive care units in 2 US level I trauma centers between May 2006 and August 2012. The study used a factorial design to test whether erythropoietin would fail to improve favorable outcomes by 20% and whether a hemoglobin transfusion threshold of greater than 10 g/dL would increase favorable outcomes without increasing complications. Erythropoietin or placebo was initially dosed daily for 3 days and then weekly for 2 more weeks (n = 74) and then the 24- and 48-hour doses were stopped for the remainder of the patients (n = 126). There were 99 patients assigned to a hemoglobin transfusion threshold of 7 g/dL and 101 patients assigned to 10 g/dL.

INTERVENTIONS Intravenous erythropoietin (500 IU/kg per dose) or saline. Transfusion threshold maintained with packed red blood cells.

MAIN OUTCOMES AND MEASURES Glasgow Outcome Scale score dichotomized as favorable (good recovery and moderate disability) or unfavorable (severe disability, vegetative, or dead) at 6 months postinjury.

RESULTS There was no interaction between erythropoietin and hemoglobin transfusion threshold. Compared with placebo (favorable outcome rate: 34/89 [38.2%; 95% CI, 28.1% to 49.1%]), both erythropoietin groups were futile (first dosing regimen: 17/35 [48.6%; 95% CI, 31.4% to 66.0%], $P = .13$; second dosing regimen: 17/57 [29.8%; 95% CI, 18.4% to 43.4%], $P < .001$). Favorable outcome rates were 37/87 (42.5%) for the hemoglobin transfusion threshold of 7 g/dL and 31/94 (33.0%) for 10 g/dL (95% CI for the difference, −0.06 to 0.25, $P = .28$). There was a higher incidence of thromboembolic events for the transfusion threshold of 10 g/dL (22/101 [21.8%] vs 8/99 [8.1%] for the threshold of 7 g/dL, odds ratio, 0.32 [95% CI, 0.12 to 0.79], $P = .009$).

CONCLUSIONS AND RELEVANCE In patients with closed head injury, neither the administration of erythropoietin nor maintaining hemoglobin concentration of greater than 10 g/dL resulted in improved neurological outcome at 6 months. The transfusion threshold of 10 g/dL was associated with a higher incidence of adverse events. These findings do not support either approach in this setting.

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Patients with severe traumatic brain injury commonly develop anemia. For patients with neurological injury, anemia is one potential cause of secondary injury, which may worsen neurological outcomes. Treatment of anemia may include transfusions of packed red blood cells or administration of erythropoietin.

Erythropoietin treatment of anemia after traumatic brain injury has the additional potential of providing neuroprotection. In experimental models, erythropoietin has improved outcome after injury. The neuroprotective mechanisms include anti-inflammatory, antiapoptotic, and vascular actions.[1,2] Multicenter trials in critically ill general trauma patients have suggested improved survival with erythropoietin administration,[3] but the effects on outcome are limited to case series and small randomized studies.[4-7] The first purpose of the trial was to assess the effect of early administration of erythropoietin on neurological outcome after injury.

Transfusions of packed red blood cells restore hematocrit and the carrying capacity of blood oxygen, but have been associated with increased risk of infection, multiorgan failure including respiratory failure, thromboembolic events, and death. Studies have shown that for most critically ill patients, there is no advantage to maintaining a higher hemoglobin concentration.[8-10]

Despite these findings in critically ill patients, concern lingers that hemoglobin concentrations as low as 7 g/dL may not be tolerated in patients with severe traumatic brain injury. Studies have either shown no difference in mortality[11] or suggested an association between transfusion and a worse neurological outcome.[12,13] A physician survey in 2009 demonstrated considerable practice variation in the use of transfusions.[14] The second purpose of this trial was to compare the effects of 2 hemoglobin transfusion thresholds on neurological recovery. The hypothesis was that the benefits of maintaining a hemoglobin concentration of 10 g/dL would exceed the risks of the transfusions required, and neurological outcome would be improved.

Methods

A randomized trial using a factorial (2 × 2) design compared administration of erythropoietin or placebo and separately compared hemoglobin transfusion thresholds (7 or 10 g/dL). The protocol (appears in Supplement 1) was approved by the US Food and Drug Administration (FDA) and by institutional review boards at each clinical site. During the first year of the study, patients were enrolled after written informed consent was obtained from their legally authorized representative. In August 2007, after approval of the requirements for emergency research, the study was conducted under regulations for the Exception From Informed Consent for Emergency Research.[15] When a family representative was subsequently located, the patient recovered sufficiently to consent, or both, he/she was asked to sign a consent form to permit continued patient participation in the study.

Patient Population

The study population included patients with a closed head injury who were not able to follow commands after resuscitation after being admitted to 1 of 2 level I trauma centers in Houston, Texas, and could be enrolled in the study within 6 hours of injury. Exclusion criteria included Glasgow Coma Scale score of 3 with fixed and dilated pupils, penetrating trauma, pregnancy, life-threatening systemic injuries, and severe preexisting disease.

Baseline Assessment

Baseline information on age, sex, and type and severity of injury were obtained on admission. Race/ethnicity was also collected as a baseline factor that might affect access to rehabilitation and other resources that could contribute to improved outcome. The race/ethnicity designation was based on information from the family or significant other, the patient, and information given about first, second, and preferred language.

The Glasgow Coma Scale score and pupillary reactivity obtained in the emergency department after resuscitation were used for the baseline neurological assessment. When patients were sedated and paralyzed at the time of assessment in the emergency department, the first unsedated examination prior to randomization was used as the enrollment neurological examination. The initial computed tomographic scan was classified using the Marshall scoring system[16]; and basal cistern compression, midline shift, and the presence of subarachnoid hemorrhage and epidural hematoma were noted.[17] The Injury Severity Score was calculated prior to randomization by the research team.[18]

Randomization and Blinding

A randomization list, stratified by study site, using 1 randomization event for both factors in blocks of 4, was prepared by the study statisticians and kept in each hospital's research pharmacy. When a new patient was enrolled, the research pharmacist prepared the study drug based on the patient's weight and treatment assignment from the randomization list and informed the investigators of the transfusion threshold assignment.

Investigators and clinical personnel caring for the patient were blinded to the study drug (erythropoietin or placebo) for each patient, but not to the transfusion threshold assignment. Personnel conducting outcome assessments were blinded to both drug treatment assignment and transfusion threshold. The clinical personnel were not provided with the outcome assessments.

Study Intervention

A detailed protocol conforming to the Guidelines for the Management of Severe Head Injury[19] was followed for the standard management of the patients (Supplement 1). Patients received 500 IU/kg of erythropoietin (Epogen, Amgen Inc) or an equal volume of saline intravenous bolus infusion over 2 minutes for each dose of the study drug. Patients received an initial dosage regimen of the assigned study drug followed by 2 additional doses, 1 per week for the next 2 weeks provided that

the patient remained in the intensive care unit and his/her hemoglobin concentration remained below 12 g/dL. For the first 74 patients, the initial dosage regimen was 1 dose given within 6 hours of injury followed by 2 additional doses given every 24 hours (erythropoietin 1 regimen). In 2009, the initial dosage regimen was changed for the subsequent 126 patients to 1 dose given within 6 hours of injury (erythropoietin 2 regimen). This change was made because of potential safety concerns raised by the FDA in the multicenter EPO Stroke Trial.[20] In that study,[20] patients who received a dosage regimen similar to the erythropoietin 1 regimen had a higher mortality rate than patients who received placebo (16.4% vs 9.0%, respectively; $P = .01$).

During the acute postinjury recovery period (until intracranial pressure monitoring and ventilatory support were no longer required), the assigned hemoglobin threshold was maintained with transfusion of leukoreduced-packed red blood cells. In patients who were actively bleeding, which may occur during the early postinjury period or during surgical procedures for intracranial injuries, hemodynamic instability was also used as an indication for transfusion in both transfusion thresholds.

Outcome Measures

The primary outcome was measured using the Glasgow Outcome Scale (GOS), which is a 5-category scale consisting of good recovery, moderate disability, severe disability, vegetative, and dead. Patients were assessed using a structured interview at 6 months after the injury.[21] The GOS score was determined either in person in a variety of settings (eg, neuropsychology office, home visit, or workplace) or over the telephone by neuropsychology personnel. Information was obtained directly from the patient, next of kin, significant other, or caretaker. If necessary, some information was obtained from records released by other facilities with appropriate consent. The GOS score was dichotomized into a prespecified favorable outcome (good recovery or moderate disability) or unfavorable outcome (severe disability, vegetative, or dead). The 3 primary safety outcomes for the transfusion threshold comparison were mortality, the incidence of adult respiratory distress syndrome (ARDS), and the incidence of infections (total number of incidences of pneumonia, bacteremia, urinary tract infection, and ventriculitis). The secondary transfusion threshold outcome was measured using the Disability Rating Scale, which is a 31-point scale ranging from 0 (no disability) to 30 (death). The secondary outcome was mortality for patients assigned to erythropoietin or placebo.

Erythropoietin Levels

Plasma and cerebrospinal fluid levels of erythropoietin were obtained prior to and 1 hour after the doses of study drug when given within 6 hours of traumatic brain injury, and at 24 and 48 hours postinjury, and then daily for the first 10 days postinjury. Erythropoietin levels were measured using a commercially available solid phase sandwich enzyme-linked immunosorbent assay (Quantikine IVD erythropoietin DEP00, R& D System Inc), which detects both native and recombinant erythropoietin to a sensitivity of 0.6 mIU/mL.

Data Analysis

An intent-to-treat statistical analysis was conducted. Baseline characteristics were compared using the Fisher exact test for categorical variables or a Wilcoxon rank sum test for continuous variables. Continuous variables were summarized using medians and quartiles. Logistic regression was used to test for an interaction for the primary outcome between the transfusion threshold and the erythropoietin dosing regimen using an α level of .10.

The primary outcome comparisons were analyzed using a 2-sample test of proportions for the study drug (1-sided test) and transfusion threshold (2-sided test). The primary futility analysis compared the erythropoietin 2 regimen with placebo ($\alpha = .15$). If we reject the null hypothesis that the percentage of favorable outcomes with the erythropoietin 2 regimen is greater than or equal to the percentage of favorable outcomes with placebo plus 20%, we conclude that studying the drug in a phase 3 trial would likely be futile. Additional details of the futility analysis are provided in the eMethods in Supplement 2.

As a secondary analysis of the GOS, drug group and transfusion threshold group were separately compared using logistic regression, adjusted for prespecified covariates of injury severity (Injury Severity Score and the International Mission for Prognosis and Analysis of Clinical Trials in [traumatic brain injury] TBI [IMPACT] probability laboratory model predictions of unfavorable outcome described by Steyerberg et al).[22] Post hoc analyses using a sliding dichotomy[23] and an ordinal logistic regression resulted in similar results and are not presented.

In the absence of evidence to the contrary, multiple imputation for missing 6-month GOS data was performed assuming data were missing at random using chained equations (mice package in R, R Foundation for Statistical Computing). The imputation was based on a logistic regression model with baseline covariates for the transfusion threshold groups, Injury Severity Score, the IMPACT laboratory model score, presence of hypoxia, the treatment group (erythropoietin vs placebo), and presence of epidural hematoma. Results were aggregated over 20 imputed sets using the variance formula by Rubin.[24]

The incidences of secondary binary outcomes were analyzed using a 2-sample test of proportions. Disability Rating Scale scores were compared using a Wilcoxon rank sum test. The Cox proportional hazard model was used to determine time-to-event hazard ratios and 95% confidence intervals. The proportional hazard assumption was examined using Schoenfeld residual plots and we tested a treatment × time interaction term. The log-rank test was used to compare survival curves. For the primary safety analysis of ARDS, 3 critical care experts independently determined whether each patient had ARDS according to the American-European consensus conference definition.[25] Cox regression analyses were performed to determine whether transfusion threshold assignment increased the risk of ARDS. Lasso-penalized Cox regression, with the penalty parameter selected by 10-fold cross-validation, was used for feature selection.[26] Censor time was defined as date of hospital discharge, withdrawal, or death, whichever oc-

curred first. Generalized estimating equations were used to compare longitudinal hemoglobin levels among treatment groups.

All analyses except the futility analysis (α = .15) and the tests of interactions for the outcomes between the transfusion threshold and the erythropoietin dosing regimen (α = .10) were conducted with an α level of .05 and 2-sided tests. All analyses were conducted using SAS version 9.3 (SAS Institute Inc), Stata version 12 (StataCorp), or R version 2.13.1 (R Foundation for Statistical Computing).

Sample Size and Power Calculations

Due to the change in the initial erythropoietin dosage regimen, the primary erythropoietin analysis plan was changed from a superiority trial to a futility trial of the erythropoietin 2 regimen group.[27] We hypothesized that 30% of patients in the placebo group would have a favorable outcome at 6 months and there would be no interaction between the erythropoietin and transfusion threshold groups. Using a 1-sided α level of .15, a sample size of 62 patients in the erythropoietin 2 regimen group, and 100 patients in the placebo group provided 91% power to test the futility hypothesis described in the analysis.

For the transfusion threshold analysis, we hypothesized that 40% of patients in the hemoglobin transfusion threshold group of 7 g/dL would have a favorable GOS score at 6 months and that there would be no interaction between the erythropoietin and transfusion threshold groups. Assuming a 2-sided test with an α level of .05, we estimated that a sample size of 200 patients, randomized in a 1:1 ratio to the 2 transfusion threshold groups, would provide 80% power to detect a 20% absolute increase in GOS score at 6 months after the injury for the hemoglobin transfusion threshold of 10 g/dL.

Results

Interaction of Randomized Factors

A statistically significant interaction between the hemoglobin transfusion threshold and erythropoietin was not detected for any reported primary, secondary, or safety outcomes; thus, the erythropoietin and placebo groups were combined for the transfusion threshold analyses and the transfusion threshold groups were combined for the erythropoietin analyses described herein.

Patient Characteristics

A total of 895 patients were screened for eligibility between May 2006 and August 2012 (**Figure 1**). Two hundred patients met eligibility criteria and were enrolled. The treatment groups had similar demographic characteristics (**Table 1**). There were no significant differences in injury characteristics between the study drug treatment groups except that prehospital hypoxia was more common in the placebo group. Except for the incidence of epidural hematoma on the admission computed tomographic scan, which was higher for the hemoglobin transfusion threshold of 10 g/dL, there were no significant differences detected in the injury characteristics between the 2 transfusion thresholds.

Adherence to Protocol and Protocol-Related Factors

Erythropoietin Protocol

All patients received the initial dose of the assigned study drug (eTable 1 in Supplement 2). The average time of the first study drug dose was 5.2 (SD, 0.8) hours after injury with 187 doses (93.5%) given within 6 hours of injury. Additional dosing information appears in eTable 1 in Supplement 2.

At enrollment prior to receiving the initial dose of study drug, the median plasma level of erythropoietin was 15.7 mIU/mL (normal range, 4-27 mIU/mL; eTable 1 in Supplement 2). In the placebo group, the median plasma erythropoietin levels gradually increased over time, peaking at 111.6 mIU/mL at 48 hours after the injury. In the patients who received erythropoietin, the median plasma levels of erythropoietin increased by 12 hours after the injury to 1745.0 mIU/mL. These elevated plasma levels of erythropoietin were sustained for a longer time in the patients receiving the erythropoietin 1 regimen compared with those receiving the erythropoietin 2 regimen (eFigure 1A in Supplement 2).

The cerebrospinal fluid levels of erythropoietin followed the same pattern (eFigure 1B in Supplement 2). At 6 hours prior to receiving the initial dose of study drug, erythropoietin was undetectable in most of the patients. In the patients receiving erythropoietin, the median cerebrospinal fluid levels of erythropoietin increased to 11.8 mIU/mL at 12 hours after the injury, and remained elevated above baseline values through 96 hours.

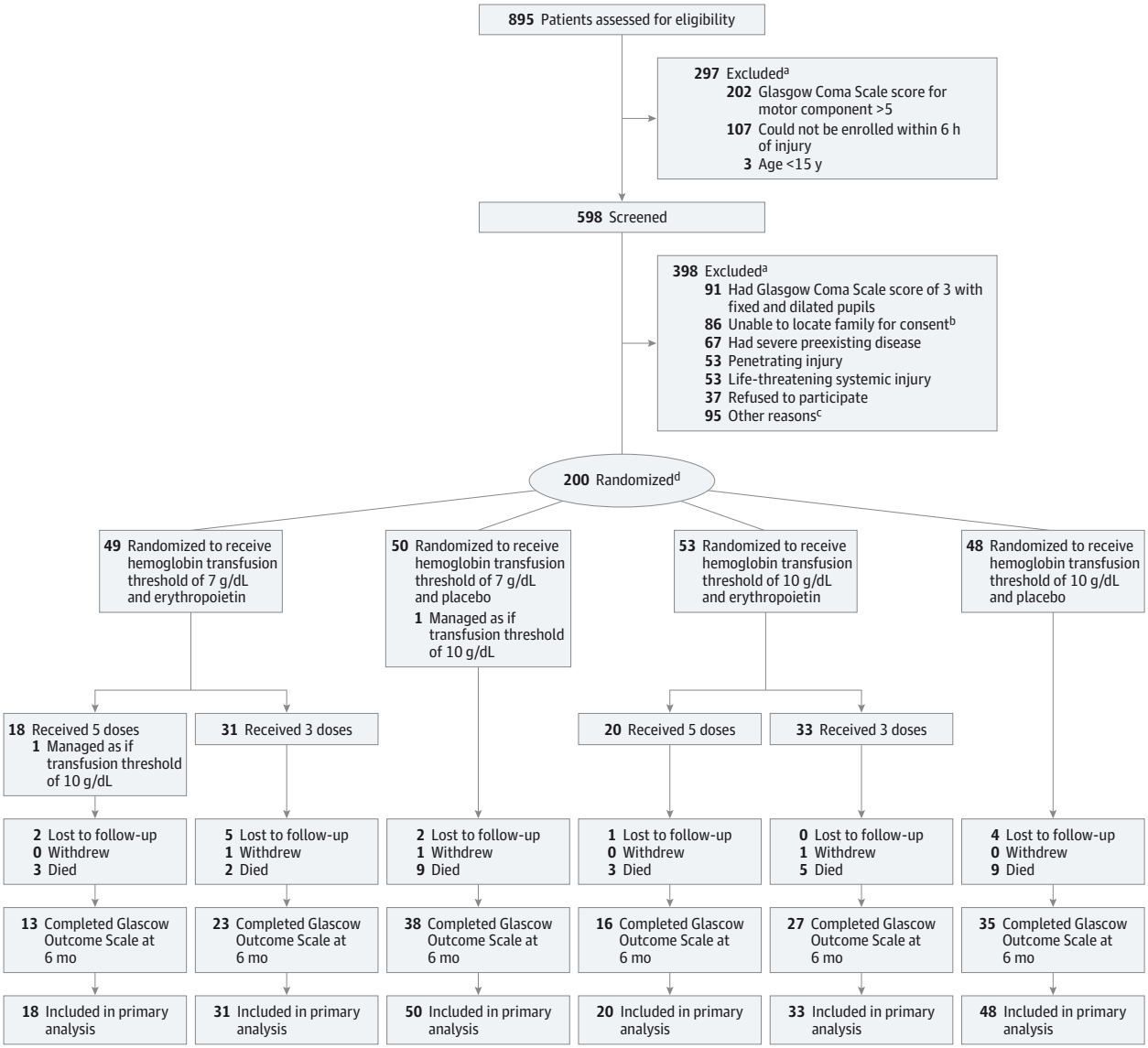
There were no differences in the number of transfusions required in the 2 erythropoietin groups. The hemoglobin concentration was less than 10 g/dL for a shorter time in the patients receiving the erythropoietin 1 regimen compared with the placebo group (**Table 2**).

Transfusion Threshold Protocol

Adherence to the protocol throughout the study was good with a few exceptions. Two patients who were assigned to the transfusion threshold of 7 g/dL were mistakenly managed as if their assigned hemoglobin threshold was 10 g/dL. In addition, there were 2 patients who were assigned to and managed according to the threshold of 7 g/dL but received a transfusion on 1 occasion not according to the protocol.

The number of units of packed red blood cells required to maintain the assigned transfusion threshold and hemoglobin concentrations over time in the treatment groups are detailed in Table 2 and **Table 3**. The number of transfusions given for active bleeding (due to traumatic injuries or during surgical procedures) was similar in the 2 transfusion groups and the major difference was in the number of transfusions required in hemodynamically stable patients to maintain the assigned hemoglobin concentration. The length of time that the hemoglobin concentration was less than 10 g/dL was higher in the group with a transfusion threshold of 7 g/dL (eFigure 2 in Supplement 2), and the average hemoglobin concentration over time was higher in the group with a transfusion threshold of 10 g/dL (Table 3).

Figure 1. Patients Screened and Enrolled in the Trial



[a] A patient could have more than 1 exclusion criteria.

[b] Exception from informed consent was in effect during 50 months and not available for 20 months of the trial.

[c] Included screened during clinical hold (n = 52), pregnant (n = 3), uncontrolled hypertension (n = 3), receiving anticoagulants (n = 1), and other reasons (n = 37).

[d] Of the 200 randomized, prospective consent was obtained for 106 and 94 had exception from informed consent.

Primary Outcome of Neurological Recovery at 6 Months

Analysis of Erythropoietin Regimens

A difference in the proportion of favorable GOS outcomes at 6 months could not be detected between patients in the placebo group enrolled during the erythropoietin 1 regimen (36%) and the erythropoietin 2 regimen (39%) (95% CI for the difference, −26.1% to 20.3%; $P = .96$). These 2 groups were combined into a single placebo group for analyses. The primary outcome was available in 35 patients (92%) enrolled during the erythropoietin 1 regimen, 57 patients (89%) during the erythropoietin 2 regimen, and 89 patients (91%) in the placebo group. The distribution of missing outcome data was similar among the 3 treatment groups ($P = .90$).

In the placebo group, 34 patients (38.2%; 95% CI, 28.1%-49.1%) recovered to a favorable outcome compared with 17 patients (48.6%; 95% CI, 31.4%-66.0%) in the erythropoietin 1 group and 17 patients (29.8%; 95% CI, 18.4%-43.4%) in the erythropoietin 2 group (**Figure 2**). The results of the logistic regression analyses in which the GOS score was adjusted for prespecified covariates and for the presence of prehospital hypoxia, which was more common in the placebo group, appear in **Table 4**. Treatment with erythropoietin was not significant in any of the models.

In the primary futility analysis, the null hypothesis was that the percentage of patients with a favorable outcome in the erythropoietin 2 regimen group would be greater than 20% plus

Table 1. Demographic and Injury Characteristics of Patients

	Factor 1[a]			Factor 2[a]	
	Erythropoietin Regimen			Hemoglobin Transfusion Threshold, g/dL	
	1[b] (n = 38)	2[b] (n = 64)	Placebo (n = 98)	7 (n = 99)	10 (n = 101)
Age, median (IQR), y	32 (23-48)	29 (23-47)	30 (22-44)	28 (21-48)	31 (24-45)
Sex, No. (%)					
Female	4 (10.5)	8 (12.5)	14 (14.3)	14 (14.1)	12 (11.9)
Male	34 (89.5)	55 (85.9)	84 (85.7)	85 (85.9)	88 (87.1)
Living as female		1 (1.6)			1 (1.0)
Race/ethnicity, No. (%)					
Asian	1 (2.6)	1 (1.6)	4 (4.1)	3 (3.0)	3 (3.0)
Hispanic	20 (52.6)	35 (54.7)	48 (49.0)	50 (50.5)	53 (52.5)
Black	7 (18.4)	10 (15.6)	26 (26.5)	20 (20.2)	23 (22.8)
White, non-Hispanic	10 (26.3)	18 (28.1)	20 (20.4)	26 (26.3)	22 (21.8)
Prehospital hypotension, No. (%)	4 (10.5)	5 (7.8)	16 (16.3)	11 (11.1)	14 (13.9)
Prehospital hypoxia, No. (%)	3 (7.9)	7 (10.9)	29 (29.6)	18 (18.2)	21 (20.8)
Mechanism of injury, No. (%)					
Assault	2 (5.3)	5 (7.8)	15 (15.3)	7 (7.1)	15 (14.9)
Fall or jump	11 (28.9)	7 (10.9)	9 (9.2)	18 (18.2)	9 (8.9)
Automobile crash	19 (50.0)	41 (64.1)	56 (57.1)	58 (58.6)	58 (57.4)
Motorcycle crash	5 (13.2)	11 (17.2)	15 (15.3)	14 (14.1)	17 (16.8)
Other	1 (2.6)	0	3 (3.1)	2 (2.0)	2 (2.0)
Injury Severity Score, median (IQR)	27 (26-35)	29 (25-37)	29 (25-38)	29 (25-38)	29 (25-35)
IMPACT probability of poor GOS score, mean (SD)	0.39 (0.3)	0.40 (0.2)	0.41 (0.3)	0.43 (0.3)	0.39 (0.3)
Motor component of GCS, No. (%)[c]					
1-3	12 (31.6)	26 (40.6)	33 (33.7)	36 (36.4)	35 (34.7)
4-5	26 (68.4)	38 (59.4)	65 (66.3)	63 (63.6)	66 (65.4)
GCS sum score, No. (%)[d]					
3-5	12 (31.6)	23 (35.9)	31 (31.6)	34 (34.3)	32 (31.7)
6-8	8 (21.1)	12 (18.8)	25 (25.5)	23 (23.2)	22 (21.8)
>8	18 (47.4)	29 (45.3)	42 (42.9)	42 (42.4)	47 (46.5)
Emergency department, No. (%)					
Pupil reactivity					
Both	25 (65.8)	41 (64.1)	55 (56.1)	63 (63.6)	58 (57.4)
1	5 (13.2)	5 (7.8)	13 (13.3)	14 (14.1)	9 (8.9)
Neither	8 (21.1)	18 (28.1)	30 (30.6)	22 (22.2)	34 (33.7)
Marshall CT scan category[16]					
Diffuse injury 1 or 2	15 (39.5)	30 (46.9)	44 (44.9)	49 (49.5)	40 (39.6)
Diffuse injury 3 or 4	14 (36.8)	10 (15.6)	22 (22.5)	23 (23.2)	23 (22.8)
Mass lesion	9 (23.7)	24 (37.5)	32 (32.7)	27 (27.3)	38 (37.6)
Present on CT scan					
Subarachnoid hemorrhage	26 (68.4)	44 (68.8)	68 (69.4)	71 (71.7)	67 (66.3)
Epidural hematoma	6 (15.8)	12 (18.8)	14 (14.3)	10 (10.1)	22 (21.8)
Hemoglobin, median (IQR), g/dL	14.7 (13.5-15.6)	14.6 (12.8-15.5)	14.2 (12.7-15.6)	14.4 (13.0-15.6)	14.6 (12.8-15.5)
Glucose, median (IQR), mmol/L	8.5 (7.2-10.7)	8.8 (7.1-10.1)	8.2 (6.9-10.1)	8.7 (7.3-10.4)	8.0 (6.7-10.0)
Surgery on admission, No. (%)	9 (23.7)	22 (34.4)	30 (30.6)	26 (26.3)	35 (34.7)
Epidural hematoma	0	3 (4.7)	6 (6.1)	3 (3.0)	6 (5.9)
Subdural hematoma	7 (18.4)	19 (29.7)	20 (20.4)	20 (20.2)	26 (25.7)
Intracerebral hematoma or contusion	2 (5.3)	0	2 (2.0)	2 (2.0)	2 (2.0)
Non-central nervous system injury	0	0	2 (2.0)	1 (1.0)	1 (1.0)

Abbreviations: CT, computed tomographic; GCS, Glasgow Coma Scale; GOS, Glasgow Outcome Scale; IQR, interquartile range.

SI conversion factor: To convert glucose to mg/dL, divide by 0.0555.

[a] This study used a 2 × 2 factorial design so the same patients were included for factors 1 and 2.

[b] Explanation of the erythropoietin 1 regimen, for the first 74 patients, the initial dosage regimen was 1 dose given within 6 hours of injury followed by 2 additional doses given every 24 hours; and for the erythropoietin 2 regimen, in 2009, the initial dosage regimen was changed for the subsequent 126 patients to 1 dose given within 6 hours of injury.

[c] Calculated at enrollment; score range is from 1 (no motor response) to 6 (follows commands).

[d] Calculated at enrollment; sum of the eye, motor, and verbal components and score range is from 3 (no response) to 15 (normal response).

the percentage in the placebo group. The null hypothesis was rejected at the α level of .15 ($P < .001$). In a similar futility analysis for the erythropoietin 1 regimen group, the null hypothesis was rejected at the α level of .15 ($P = .13$). It is unlikely that either dosage regimen for erythropoietin has at least a 20% higher favorable outcome compared with the placebo group.

Analysis of Hemoglobin Transfusion Thresholds

The 6-month GOS score was available for 87 patients (87.9%) in the hemoglobin transfusion threshold group of 7 g/dL and 94 patients (93.1%) in the threshold group of 10 g/dL (Figure 2). The distribution of missing outcome data was similar among the 2 transfusion threshold groups (odds ratio [OR],

Table 2. Transfusion Characteristics

	Factor 1[a]			Factor 2[a]	
	Erythropoietin Regimen			Hemoglobin Transfusion Threshold, g/dL	
Transfusion Characteristics	1[b] (n = 38)	2[b] (n = 64)	Placebo (n = 98)	7 (n = 99)	10 (n = 101)
Packed red blood cells					
Patients with at least 1 unit required, No. (%)	23 (60.5)	39 (60.9)	63 (64.3)	52 (52.5)	73 (72.3)
No. of units required	162	195	407	243	521
Mean per patient (range)	7.0 (1-15)	5.0 (1-17)	6.5 (1-22)	4.7 (1-22)	7.1 (1-21)
No. of units required to keep hemoglobin above assigned threshold	83	109	228	87	333
Mean per patient (range)	4.4 (1-7)	3.8 (1-11)	3.9 (1-16)	2.4 (1-5)	4.7 (1-16)
No. of units required due to active bleeding	79	82	167	144	184
Mean per patient (range)	5.3 (1-11)	3.2 (1-10)	4.3 (1-18)	3.8 (1-18)	4.4 (1-12)
No. of units required after acute care per clinical decision	0	4	8	8	4
Mean per patient (range)	0	2 (2-2)	2 (1-3)	2 (2-2)	2 (1-3)
No. of units given in violation of protocol	0	0	4	4	0
Mean per patient (range)	0	0	2 (2-2)	2 (2-2)	0
Period that hemoglobin was <10 g/dL, median (IQR), h	8.3 (0.3-16.4)[c]	13.4 (3.5-39.7)	18.9 (5.7-48.1)	33.9 (4.0-60.8)	10.5 (1.1-19.0)[c]
Period that hemoglobin was <7 g/dL, median (IQR), h	0	0	0 (0-0.2)	0 (0-0.6)	0[d]

Abbreviation: IQR, interquartile range.

[a] This study used a 2 × 2 factorial design so the same patients were included for factor 1 and factor 2.

[b] Explanation of the erythropoietin 1 regimen, for the first 74 patients, the initial dosage regimen was 1 dose given within 6 hours of injury followed by 2 additional doses given every 24 hours; and for the erythropoietin 2 regimen, in 2009, the initial dosage regimen was changed for the subsequent 126 patients to 1 dose given within 6 hours of injury.

[c] Significantly different compared with placebo group ($P = .02$).

[d] Significantly different from 7-g/dL hemoglobin transfusion threshold group ($P < .001$).

Table 3. Hemoglobin Concentrations Over Time

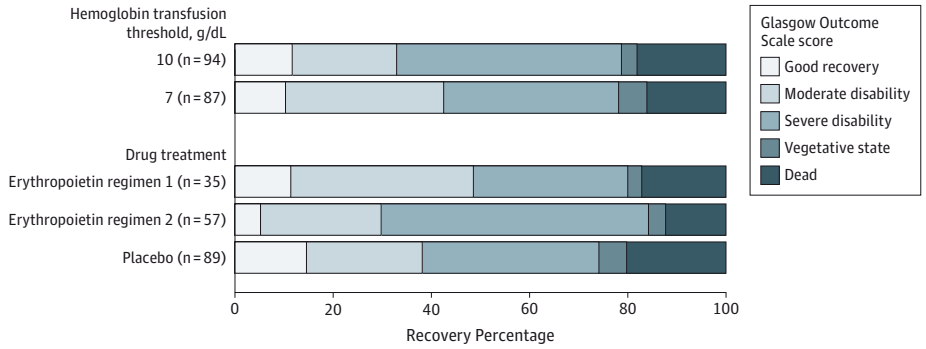
	Median (IQR) Hemoglobin Concentrations by Time After Injury, g/dL				
Treatment Group	Enrollment	Day 9	Day 16	Day 23	Day 30
Factor 1[a]					
Erythropoietin regimen					
1[b]	(n = 38) 14.7 (13.5-15.6)	(n = 35) 10.9 (10.3-12.3)	(n = 27) 11.0 (9.4-12.1)	(n = 16) 11.6 (10.9-12.3)	(n = 11) 11.6 (11.3-12.3)
2[b]	(n = 64) 14.6 (12.8-15.5)	(n = 57) 10.6 (9.5-11.9)	(n = 47) 10.6 (9.1-11.7)	(n = 36) 11.2 (10.3-12.8)	(n = 23) 10.8 (9.7-12.2)
Placebo	(n = 98) 14.2 (12.7-15.6)	(n = 80) 10.9 (9.4-11.7)	(n = 66) 10.4 (9.6-11.8)	(n = 49) 10.9 (9.8-12.1)	(n = 34) 11.5 (9.7-12.2)
Factor 2[a]					
Hemoglobin transfusion threshold, g/dL					
7	(n = 99) 14.4 (13.0-15.6)	(n = 85) 9.7 (8.6-10.9)	(n = 65) 9.6 (8.8-10.6)	(n = 48) 10.7 (9.6-11.5)	(n = 28) 10.8 (9.5-11.5)
10	(n = 101) 14.6 (12.8-15.5)	(n = 87) 11.4 (10.7-12.2)	(n = 75) 11.2 (10.4-12.2)	(n = 53) 11.9 (10.9-12.8)	(n = 40) 11.7 (10.8-12.4)

Abbreviation: IQR, interquartile range.

[a] This study used a 2 × 2 factorial design so the same patients were included for factor 1 and factor 2.

[b] Explanation of the erythropoietin 1 regimen, for the first 74 patients, the initial dosage regimen was 1 dose given within 6 hours of injury followed by 2 additional doses given every 24 hours; and for the erythropoietin 2 regimen, in 2009, the initial dosage regimen was changed for the subsequent 126 patients to 1 dose given within 6 hours of injury.

Figure 2. Glasgow Outcome Scale Scores at 6 Months for Complete Cases



For the primary outcome, good recovery and moderate disability were combined as a favorable outcome. Severe disability, vegetative, and dead were combined as an unfavorable outcome. For the first 74 patients, the initial dosage regimen was 1 dose given within 6 hours of injury followed by 2 additional doses given every 24 hours (erythropoietin 1 regimen). In 2009, the initial dosage regimen was changed for the subsequent 126 patients to 1 dose given within 6 hours of injury (erythropoietin 2 regimen).

1.85 [95% CI, 0.64 to 5.80]; P = .24). Thirty-seven patients (42.5%) assigned to the transfusion threshold of 7 g/dL recovered to a favorable outcome compared with 31 patients (33.0%) assigned to the transfusion threshold of 10 g/dL (95% CI for difference, −0.06 to 0.25). In the primary analysis using multiple imputation of missing GOS scores, there was no significant difference in outcome detected between the 2 threshold groups (95% CI for difference, −0.07 to 0.20; P = .34).

After adjustment for prespecified covariates (Table 4), an association between transfusion threshold and GOS outcome was not detected (OR, 0.75 [95% CI, 0.36-1.55]; P = .43). In a post hoc analysis adjusting for incidence of epidural hematoma as an additional covariate in the logistic regression model, an association between transfusion threshold and GOS outcome was also not detected (OR, 0.61 [95% CI, 0.28-1.30]; P = .20).

Secondary Outcome of Disability Rating Scale Score

The median 6-month Disability Rating Scale score was 5 (interquartile range [IQR], 1.25-12.75) in the erythropoietin 1 regimen (P = .52 vs placebo), 7 (IQR, 4-12) in the erythropoietin 2 regimen (P = .97 vs placebo), and 6.5 (IQR, 3-18.75) in the placebo group. The median 6-month score was 5 (IQR, 2.25-9.75) with the transfusion threshold of 7 g/dL and 8 (IQR, 4-17) with 10 g/dL (P = .06). A higher score represents a worse outcome.

Safety and Secondary Outcomes

Analysis of Mortality

Information about survival to 6 months was available for 190 patients (95%) enrolled in the study. Six patients in the erythropoietin 1 regimen group, 7 in the erythropoietin 2 regimen group, and 18 in the placebo group died during the 6 months of follow-up. Kaplan-Meier survival curves for the erythropoietin 1 regimen group (P = .75) and for the erythropoietin 2 regimen group (P = .25) were not significantly different from the placebo group (**Figure 3**).

There were 14 deaths during the 6 months of follow-up with the transfusion threshold of 7 g/dL and 17 with the threshold of 10 g/dL. Kaplan-Meier survival curves for the 2 transfusion threshold groups are illustrated in **Figure 4**. The overall log-rank test was not significant (P = .72).

Analysis of ARDS

A total of 16 patients (16.2%) with the transfusion threshold of 7 g/dL and 25 patients (24.7%) the threshold of 10 g/dL developed ARDS (P = .16). In the final Cox regression model (**Table 5**), the transfusion threshold of 10 g/dL was not significantly associated with ARDS (hazard ratio, 1.79 [95% CI, 0.93-3.45]; P = .08).

Analysis of Infections

The most common infection was pneumonia, which occurred in 33 patients (17%). The second most common infection was urinary tract infection, which occurred in 21 patients (11%), followed by ventriculitis and bacteremia. There were a total of 27 patients with the transfusion threshold of 7 g/dL who had 1 or more infectious complications and 36 patients with the threshold of 10 g/dL (95% CI for difference in proportions, −0.22 to 0.05, P = .26).

Analysis of Thromboembolic Events

The incidence of thromboembolic events was examined because a higher overall incidence was observed with the transfusion threshold of 10 g/dL and a higher incidence of upper extremity deep venous thrombosis (DVT) was found in the groups treated with erythropoietin (eTable 2 in Supplement 2).

A total of 30 patients developed 1 or more thromboembolic events during the 6 months of follow-up. The majority of thromboembolic events occurred 3 days after the injury; 3 events occurred 30 days after the injury. Of the 200 patients, 25 (12.5%) developed DVT. Nine patients (4.5%) developed pulmonary embolus. Four patients had multiple thromboembolic events. The patients with the transfusion threshold of 10 g/dL had a significantly greater incidence of 1 or more thromboembolic events (22 patients [21.8%] vs 8 patients [8.1%] with the transfusion threshold of 7 g/dL; OR, 0.32

Table 4. Primary Outcome Adjusted for Prespecified Covariates and Baseline Variables

	Multiple Imputation Pooled Estimates, OR (95% CI)	*P* Value
Factor 1[a]		
Erythropoietin 1 regimen[b]		
Logistic regression model adjusted for prespecified covariates		
Intercept	9.09 (1.44-57.52)	.02
Compared with placebo	1.56 (0.60-4.08)	.36
Injury Severity Score[c]	0.99 (0.93-1.04)	.58
IMPACT probability of unfavorable GOS score per 10% unit increment[c]	0.55 (0.44-0.69)	<.001
Logistic regression model adjusted for prespecified covariates and baseline variables that were not balanced		
Intercept	8.84 (1.39-56.41)	.01
Compared with placebo	1.78 (0.66-4.83)	.24
Injury Severity Score[c]	0.99 (0.93-1.04)	.51
IMPACT probability of unfavorable GOS score per 10% unit increment[c]	0.52 (0.41-0.66)	<.001
Prehospital hypoxia present[d]	2.28 (0.64-8.06)	.16
Erythropoietin 2 regimen[b]		
Logistic regression model adjusted for prespecified covariates		
Intercept	9.48 (1.58-57.03)	.01
Compared with placebo	0.63 (0.27-1.48)	.29
Injury Severity Score[c]	0.99 (0.94-1.04)	.63
IMPACT probability of unfavorable GOS score, per 10% unit increment[c]	0.53 (0.41-0.67)	<.001
Logistic regression model adjusted for prespecified covariates and baseline variables that were not balanced		
Intercept	9.73 (1.62-58.56)	.01
Compared with placebo	0.69 (0.29-1.65)	.40
Injury Severity Score[c]	0.99 (0.94-1.04)	.60
IMPACT probability of unfavorable GOS score per 10% unit increment[c]	0.50 (0.38-0.64)	<.001
Prehospital hypoxia present[d]	2.55 (0.78-8.28)	.12
Factor 2[a]		
Hemoglobin transfusion threshold analysis		
GOS logistic regression model adjusted for prespecified covariates		
Intercept	11.10 (2.10-58.60)	.005
10 vs 7 g/dL transfusion threshold	0.75 (0.36-1.55)	.43
Injury Severity Score[c]	0.98 (0.94-1.03)	.43
IMPACT probability of poor outcome per 10% unit increment[c]	0.54 (0.44-0.66)	<.001
GOS logistic regression adjusted model adjusted for prespecified covariates and baseline variables that were not balanced		
Intercept	11.47 (2.06-63.84)	.006
10 vs 7 g/dL hemoglobin transfusion threshold	0.61 (0.28-1.30)	.20
Injury Severity Score[c]	0.98 (0.93-1.02)	.33
IMPACT probability of poor outcome per 10% unit increment[c]	0.54 (0.44-0.67)	<.001
Presence of epidural hematoma[d]	4.74 (1.63-13.73)	.004

Abbreviations: GOS, Glasgow Outcome Scale; IMPACT, International Mission for Prognosis and Analysis of Clinical Trials in [traumatic brain injury] TBI; OR, odds ratio.

[a] This study used a 2 × 2 factorial design.

[b] Explanation of the erythropoietin 1 regimen, for the first 74 patients, the initial dosage regimen was 1 dose given within 6 hours of injury followed by 2 additional doses given every 24 hours; and for the erythropoietin 2 regimen, in 2009, the initial dosage regimen was changed for the subsequent 126 patients to 1 dose given within 6 hours of injury.

[c] Indicates prespecified covariate.

[d] Indicates baseline covariate that was not balanced.

[95% CI, 0.12-0.79], *P* = .009). No statistically significant differences for other adverse events except anemia could be detected between the 2 transfusion thresholds (eTable 2 in Supplement 2).

During the first 30 days after injury, DVT occurred in 5 patients (13.2%) in the erythropoietin 1 regimen group, 11 (17.1%) in the erythropoietin 2 regimen group, and 7 patients (7.1%) in the placebo group. The incidence of the subcategory of upper extremity DVT was significantly higher in the erythropoietin 2 regimen group compared with the placebo group (OR, 13.7 [95% CI, 1.76-619.09]; *P* = .003). Pulmonary embolus occurred in none of the patients in the erythropoietin 1 group, but in 4 patients (6.3%) in the erythropoietin 2 group, and 3 patients (3.1%) in the placebo group. The incidence of other cardiovascular complications was also significantly higher in the erythropoietin 1 group than in the placebo group (OR, 10.6 [95% CI, 1.89-109.9], *P* = .002; eTable 2 in Supplement 2).

Discussion

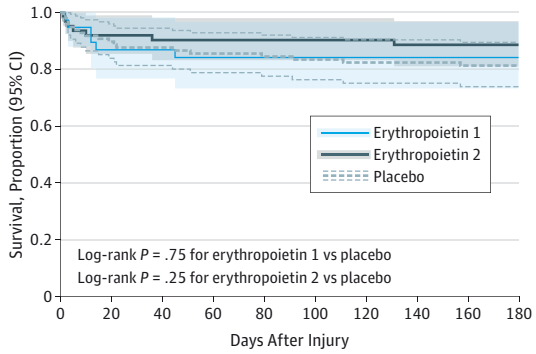
Maintaining a hemoglobin concentration of approximately 10 g/dL has long been a management strategy to improve cerebral oxygenation in patients with traumatic brain injury. In studies of patients with traumatic brain injury and anemia,[28,29] hemoglobin transfusion does improve brain oxygenation in some patients. Other potentially beneficial effects of maintaining a higher hemoglobin concentration are to avoid increased intracranial pressure induced by anemia,[30] and to provide a higher blood pressure and therefore better cerebral perfusion pressure.

This transfusion practice was expected to reduce neurological injury, particularly during the acute recovery period when the brain is most vulnerable to ischemic insults. However, in this study, no long-term benefit on neurological outcome was detected with the hemoglobin transfusion threshold of 10 g/dL, and a greater incidence of thromboembolic events was observed with this threshold.

There were several limitations in the design of the study. The trial was powered for a relatively large difference in outcome for the transfusion threshold factor because it was thought that maintaining an adequate oxygen delivery to the injured brain was an important critical care principle for patients with traumatic brain injury. A small decrease in the percentage of favorable outcomes with either transfusion threshold cannot be excluded by the results. However, it is unlikely that an increase in the percentage of favorable outcomes with the 10 g/dL transfusion threshold would have been detected even with a larger sample size.

The trial was conducted at only 2 clinical sites, which could limit the ability to generalize the results, and required 6 years to complete enrollment. Two additional factors contributed to

Figure 3. Kaplan-Meier Survival Curves for the Erythropoietin Dosing Regimen Groups

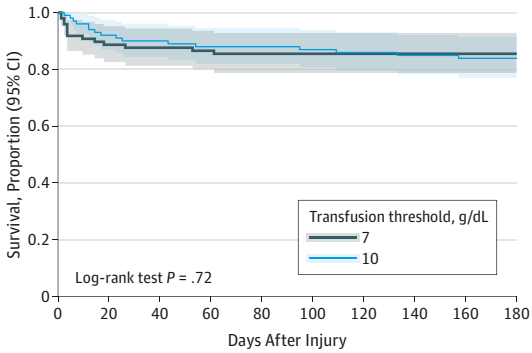


No. at risk

Erythropoietin regimen					
1	38	32	31		30
2	64	54	54		53
Placebo	98	83	81		74

For the first 74 patients, the initial dosage regimen was 1 dose given within 6 hours of injury followed by 2 additional doses given every 24 hours (erythropoietin 1 regimen). In 2009, the initial dosage regimen was changed for the subsequent 126 patients to 1 dose given within 6 hours of injury (erythropoietin 2 regimen).

Figure 4. Kaplan-Meier Survival Curves for the Hemoglobin Transfusion Threshold Groups



No. at risk

Tranfusion threshold					
7 g/dL	99	82	80		77
10 g/dL	101	87	86		80

Table 5. Cox Proportional Hazard Model for Adult Respiratory Distress Syndrome

	No. of Patients	No. of Events	HR (95% CI)	*P* Value
Hemoglobin transfusion threshold, g/dL				
10	101	25	1.79 (0.93-3.45)	.08
7	99	16	1 [Reference]	
GCS sum score per 1 unit of increment[a]			0.84 (0.74-0.96)	.01
ISS per 1 unit of increment			1.06 (1.03-1.10)	<.001
Hypotension				
Yes	25	5	0.60 (0.23-1.59)	.30
No	175	36	1 [Reference]	
Place of intubation				
Emergency department	152	34	3.48 (1.40-8.65)	.007
ICU or field (reference)	48	7	1 [Reference]	
CT category				
High risk	111	21	0.61 (0.32-1.19)	.15
Low risk	89	20	1 [Reference]	

Abbreviations: CT, computed tomographic; GCS, Glasgow Coma Scale; HR, hazard ratio; ICU, intensive care unit; ISS, Injury Severity Score.

[a] Indicates sum of the eye, motor, and verbal components at enrollment.

the lengthy recruitment. First, enrollment under the Exception From Informed Consent[15] was not allowed in the early months of the study, and it was difficult to recruit patients within the 6-hour window. Second, the trial was on clinical hold for approximately 1 year due to safety concerns about the initial erythropoietin dosage regimen. There were no changes in patient management at the 2 sites during the period of the trial, but systematic changes in patient characteristics cannot be excluded.

Translating preclinical studies with erythropoietin to a clinical trial design had some limitations. The effective time window for erythropoietin neuroprotection is 6 hours in experimental traumatic brain injury.[31,32] This timeframe is feasible clinically and almost all enrolled patients received their first dose of study drug within 6 hours of injury. However, the dose of erythropoietin that is safe in patients is at the lower end of the dosage range that has been found to be effective in rodent models of injury. The most effective erythropoietin dose in experimental models (5000 IU/kg) is 10 times the dose used in this study.[33]

In addition, an initial dosage regimen of 3 daily doses has been more effective than a single initial dose in experimental studies.[34] Based on the experience of a multicenter stroke trial reported in 2008,[20] there was concern by the FDA that the initial regimen of 3 daily doses of erythropoietin (erythropoietin 1 dose regimen) would impose a greater risk of death. This concern resulted in a modified study design after approximately one-third of the patients had been enrolled in the trial. We did not detect an increased mortality rate with the erythropoietin 1 dose regimen, and the neurological outcome results were more promising than with the erythropoietin 2 dose regimen. However, because this dose regimen was stopped early, the numbers of cases are too small to draw any conclusions.

Conclusions

Among patients with closed head injury, neither the administration of erythropoietin nor maintaining hemoglobin concentration of at least 10 g/dL resulted in improved neurological outcome at 6 months. These findings do not support either approach in patients with traumatic brain injury.

ARTICLE INFORMATION

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