Supplementary Online Content

Robertson CS, Hannay HJ, Yamal J-M, Gopinath S, Goodman JC, Tilley BC, and the Epo Severe TBI Trial Investigators. Effect of erythropoietin administration and transfusion threshold on neurological recovery after traumatic brain injury: a randomized clinical trial. *JAMA*. doi:10.1001/jama.2014.6490

This supplementary material has been provided by the authors to give readers additional information about their work

eMethods

Statistical Methods

Explanation of the futility hypotheses and analysis

The futility analysis was based on previous work in stroke and Parkinson's disease.[1] The futility hypotheses were as follows:

$H_0: p_{EPO_2} - p_{PLC} \geq \Delta$ versus $H_A: p_{EPO_2} - p_{PLC} < \Delta$

p_{EPO_2} and p_{PLC} are the proportions of participants expected to have a favorable GOS outcome in the treated group under dosing regimen 2 and placebo group, respectively, and $\Delta = 0{\cdot}2$ denotes the 20% increase in favorable outcomes over placebo considered clinically meaningful.

As a secondary exploratory analysis, we also conducted the futility analysis for an increase of 10% in the percentage of favorable outcomes. The null hypothesis was rejected for the Epo 2 regimen group (p=0.01) but not for the Epo 1 regimen group (p=0.47). It is unlikely that the Epo 2 regimen has even a 10% improvement in outcome over the percent of favorable outcomes in placebo, but the Epo 1 regimen was not shown to be futile for a 10% higher percentage of favorable outcomes compared to the placebo group.

References:

1. Palesch YY, Tilley BC, Sackett DL, Johnson KC, Woolson R. Applying a phase II futility design to therapeutic stroke trials. *Stroke* 2005:36:2410-2014.

Definitions for Expected Adverse Events

Pneumonia[1]: A new or progressive chest x-ray finding of infiltrate, consolidation, cavitation, or pleural effusion, plus any of the following:

- New onset of purulent sputum or change in character of sputum
- Isolation of organism from blood culture
- Isolation of pathogen from specimen obtained by transtracheal aspiration, bronchial brushing, or biopsy
- Isolation of virus or detection of viral antigen in respiratory secretions
- Diagnostic single antibody titer (IgM) or fourfold increase in paired serum samples (IgG) for pathogen
- Histopathological evidence of pneumonia

Urinary tract infection[1]: must meet at least one of the following criteria:

- At least one of the following signs or symptoms with no other recognized cause: fever (.38° C), urgency, frequency, dysuria, or suprapubic tenderness and patient has a positive urine culture, that is, $\geq 10^5$ microorganisms per cm^3 or urine with no more than two species of microorganisms OR
- At least two of the following signs or symptoms with no other recognized cause: fever (>38°C), urgency, frequency, dysuria, or suprapubic tenderness and at least one of the following:
  - positive dipstick for leukocyte esterase and/or nitrate
  - pyuria (urine specimen with >10 WBC/mm^3 or >3 WBC/high power field of unspun urine)
  - organisms seen on Gram stain of unspun urine
  - at least two urine cultures with repeated isolation of the same uropathogen (gram-negative bacteria or S. saprophyticus) with $\geq 10^2$ colonies/ml in nonvoided specimens
  - $\leq 10^5$ colonies/ml of a single uropathogen (gram-negative bacteria or S. saprophyticus) in a patient being treated with an effective antimicrobial agent for a urinary tract infection
  - physician diagnosis of a urinary tract infection
  - physician institutes appropriate therapy for a urinary tract infection

Ventriculitis[1]: The finding of positive CSF culture or fever, headache, stiff neck, meningeal signs, cranial nerve signs, or irritability, plus

- Physician institutes anti-microbial therapy, plus
- At least one of the following:
  - Increased WBC's, increased protein and/or decreased glucose in CSF
  - Positive gram stain of CSF
  - Positive blood culture
  - Positive antigen test of blood, CSF, or urine
  - Diagnostic single antibody titer (IgM) or fourfold increase in paired serum samples (IgG) for pathogen

Bacteremia: The finding of a positive blood culture of a pathogenic organism.

ARDS (Adult Respiratory Distress Syndrome)[2]*:* Pulmonary edema caused by increased pulmonary capillary permeability, not by increased pulmonary hydrostatic pressure (i.e., in the absence of left heart failure). The criteria developed by consensus conference (2) include:

Bilateral infiltrates on chest x-ray
Hypoxia (PaO_2/FiO_2 < 200)
Normal PWP or no clinical findings of left heart failure on physical exam

SIRS (Systemic Inflammatory Response Syndrome) [3]*:* The complex findings that result from a systemic activation of the innate immune response, regardless of the cause. SIRS is considered to be present when patients have all four of the following:
Temperature >38°C or <36°C
Heart rate >90 beats/min
Hyperventilation, with respiratory rate >20 breaths/min or $PaCO_2$ < 32 mm Hg
WBC > 12,000 or < 4000 or >10% immature forms

Sepsis[3]*:* SIRS plus infection.

*MODS (*Multiple Organ Dysfunction Syndrome) [4,5]*:* Sepsis with organ dysfunction. The peak SOFA (Sepsis-related Organ Failure Assessment) score during the hospitalization was used to describe this condition.

Septic shock[3]*:* Sepsis with arterial hypotension despite adequate fluid resuscitation.

Brain tissue hypoxia: $PbtO_2$ < 10 mmHg for at least 1 hour

Deep venous thrombophlebitis (DVT): The clinical findings of a swollen, tender, erythematous extremity or of fever plus diagnostic findings on duplex Doppler ultrasound or on venography.

Pulmonary embolus (PE): One or more of the following clinical findings:
dyspnea, tachypnea
pleuritic chest pain
hemoptysis
syncope
hypoxia
EKG changes of right heart strain (RBBB, right axis deviation, S1Q3T3 pattern)
plus a high probability lung scan
or a positive CT angiogram or pulmonary angiogram
or pathological findings of a PE

Acute myocardial infarction (acute MI): One of the following biochemical markers of myocardial injury:
typical rise and fall of troponin
rapid rise and fall of CK-MB
and at least one of the following clinical findings:
chest pain x 30 minutes without relief from nitrates
development of pathological q waves on EKG
new ST elevation (>1mm) in 2 contiguous leads
new ST elevation (>2mm) in precordial leads
new LBBB
EKG changes of posterior infarction (ST depression of 2mm in V1V2)
or pathological findings of an acute MI

Severe hypertension: MAP > 130 mmHg for at least 1 hour despite anti-hypertensive treatment

References:

1. CDC definitions for nosocomial infections, 1988. *Am Rev Respir Dis* 1989; 139:1058-1059
2. Bernard GR, Artigas A, Brigham KL, Carlet J, Falke K, Hudson L, Lamy M, Le GJ, Morris A, Spragg R: Report of the American-European Consensus conference on acute respiratory distress syndrome: definitions, mechanisms, relevant outcomes, and clinical trial coordination. Consensus Committee. *J Crit Care* 1994; 9:72-81
3. Bone RC, Balk RA, Cerra FB, Dellinger RP, Fein AM, Knaus WA, Schein RM, Sibbald WJ: Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. The ACCP/SCCM Consensus Conference Committee. American College of Chest Physicians/Society of Critical Care Medicine. *Chest* 1992; 101:1644-1655
4. Vincent JL, Moreno R, Takala J, Willatts S, De Mendonca A, Bruining H, Reinhart CK, Suter PM, Thijs LG: The SOFA (Sepsis-related Organ Failure Assessment) score to describe organ dysfunction/failure. On behalf of the Working Group on Sepsis-Related Problems of the European Society of Intensive Care Medicine. *Intensive Care Med* 1996; 22:707-710
5. Peres BD, Melot C, Lopes FF, Nguyen B, V, Vincent JL: The Multiple Organ Dysfunction Score (MODS) versus the Sequential Organ Failure Assessment (SOFA) score in outcome prediction. *Intensive Care Med* 2002; 28:1619-1624

Justification of the Exception From Informed Consent

The study meets the criteria for exemption from informed consent requirements for emergency research under 21 CFR 50.24:

1. **TBI is life-threatening disorder with no effective treatment:** Patients with severe TBI who will be eligible for this study have a life-threatening condition with no satisfactory alternative treatment, and the proposed randomized trial is necessary to determine the effectiveness of the treatment with erythropoietin (Epo) and also to determine the optimal transfusion trigger for this patient group.

2. **Obtaining written informed consent is not feasible:** Obtaining informed consent for this study is not feasible because the patients eligible for the study will not be able to give informed consent, the treatment with Epo must be given within 6 hr of injury in order to be effective and surrogates for informed consent are not likely to be available at the hospital within this time frame for a large percentage of the eligible patients, and there is no way to identify prospectively the patients that will be eligible for the study.

3. **Epo study has potential direct benefit to patients:** Participation in the study has potential direct benefit for the patients. All patients enrolled in the trial will receive standard management of their head injury. Experimental studies in models of traumatic brain injury have shown significant improvements in outcome with erythropoietin treatment.

4. **Epo study has reasonable risk relative to medical condition of patients:** The risk/benefit ratio of the study is favorable, considering the high mortality/morbidity of severe TBI. The risk of Epo (with the target hemoglobin levels planned in this study) for trauma patients who are critically ill has been quite low, consisting primarily of an increased risk of thrombophlebitis. The potential adverse effects in other populations with administration of Epo include hypertension, increased mortality and cardiovascular adverse events, and tumor progression in patients with cancer. These increased risks have primarily occurred in studies where a hemoglobin concentration greater than 12 g/dl has been targeted, and in patients with chronic renal failure or cancer. In addition, an increased incidence of thrombophlebitis was observed post-op in orthopedic patients. To minimize these risks, our study excludes patients with uncontrolled hypertension, with significant preexisting cardiovascular disease, chronic renal disease, and with active cancer. In addition, the maximum hemoglobin concentration targeted in the study is 10 g/dl, and the study uses the recommended practice of holding the weekly doses of study drug if the hemoglobin concentration is greater than 12 g/dl or has increased more than 1g/dl over 2 weeks.

5. **Time window for Epo neuroprotection:** The therapeutic window for neuroprotection with Epo has been studied in experimental models. Like most neuroprotective agents, the effectiveness of Epo is best when given very early after injury (2-3 hours after injury), but still has some significant neuroprotective effects as late as 6 hours after injury. By 9 hours post-injury, there is no significant neuroprotective effect with Epo administration.

In addition to these acute neuroprotective effects, there are a few studies that suggest that other beneficial effects of Epo (including neurogenesis and angiogenesis) may occur and improve neurobehavioral outcome after stroke and trauma even when given 24 hr after the neurological injury.

6. **Epo study is impracticable without exception:** The proposed study is impracticable without waived consent for two reasons. First, we may not be able to enroll a sufficient number of patients to complete the study in a reasonable amount of time using prospective written consent, and secondly we are not able to enroll any patients within the time frame that is probably needed to have optimal neuroprotection with Epo using prospective written consent.

eTable 1. Compliance with Epo Dosing Regimens and Median Plasma Epo Levels Before and One Hour After Doses

	Enrollment	24 hours	48 hours	Day 9	Day 16
Number of Patients Receiving Doses					
Epo 1 regimen	38	37[a]	38	22	16
Epo 2 regimen	64			34	26
Placebo	98	36	36	41	30[b]
Median (25th, 75th percentiles) Plasma Epo Levels (mIU/ml)					
Epo 1 – pre dose	15·34 (.3,63.6)	1657·58 (1235.0,2033.5)	1569·20 (964.1,1862.8)		
Epo 1 – post dose	1923·87 (1401.3,2561.6)	1951·37 1616.8,2507.0	1909·90 1218.8,2549.1		
Epo 2 – pre dose	16·86 (4.5, 28.0)	1561·46 (1336.4,1909.5)	758·06 (446.7,1033.0)		
Epo 2 – post dose	1971·24 1630.8,2444.7				
Placebo – pre dose	15·41 (2.7, 40.1)	64·37 139.8,161.1	111·56 (52.2,213.7)		
Placebo – post dose	19·10 5.9,50.6	78·89 32.6,148.1	81·13 40.3,304.7		

[a] = All patients except one received the complete initial dose regimen (single dose in Epo 2 regiment group, dose every 24 hours for three doses in Epo 1 regimen group). One Epo 1 regiment patient did not receive the 24 hour dose of Epo.

[b] = Two patients randomized to the placebo group received a dose of Epo rather than saline on day 16 due to pharmacy error.

eTable 2. Complications During First 30 Days After Injury

	Factor 1 (Transfusion Threshold)		Factor 2 (Epo Study Drug)		
	Transfusion Threshold 7 g/dl N=99 N (%)	**Transfusion Threshold 10 g/dl N=101 N (%)**	**Epo 1 Regimen N=38 N (%)**	**Epo 2 Regimen N=64 N (%)**	**Placebo N=98 N (%)**
Central Nervous System (CNS) Complications					
Intracranial hypertension requiring second or third tier therapy	39 (39)	43 (43)	16 (42)	23 (36)	43 (44)
Brain tissue hypoxia	31 (31)	26 (26)	12 (32)	16 (25)	29 (30)
Delayed/recurrent intracranial hematoma	24 (24)	37 (37)	16 (42)	16 (25)	29 (30)
Seizure	4 (4)	7 (7)	3 (8)	3 (5)	5 (5)
Hydrocephalus	4 (4)	2 (2)	2 (5)	2 (3)	2 (2)
Stroke	1 (1)	2 (2)	3 (8)	--	--
CSF leak	1 (1)	4 (4)	2 (5)	1 (2)	2 (2)
Subgaleal fluid collection	1 (1)	1 (1)	1 (3)	0	1 (1)
Pneumocephalus	0	2 (2)	1 (3)	1 (2)	0
Chronic subdural hematoma	2 (2)	0	1 (3)	1 (2)	0
Carotid cavernous fistula	1 (1)	0	0	1 (2)	0
Cardiovascular Complications					
Hypotension	32 (32)	28 (28)	13 (34)	20 (31)	27 (28)
Thrombophlebitis – deep venous	7 (7)	16 (16)	5 (13)	11 (17)	7 (7)
Jugular venous	1 (1)	3 (3)	2 (5)	--	2 (2)
Lower extremity venous	2 (2)	6 (6)	1 (3)	3 (5)	4 (4)
Upper extremity venous	4 (4)	7 (7)	2 (5)	8 (13)*	1 (1)
Pulmonary embolus	1 (1)	6 (6)		4 (6)	3 (3)
Cardiac arrest with CPR	2 (2)	3 (3)	2 (5)	1 (2)	2 (2)
Acute myocardial infarction	1 (1)	1 (1)	--	1 (2)	1 (1)
Other cardiovascular complications[a]	6 (6)	6 (6)	7 (18)*	2 (3)	3 (3)
Gastrointestinal Complications					
Elevated transaminases	25 (25)	26 (26)	11 (29)	14 (22)	26 (27)
Other GI complications	2 (2)	6 (6)	2 (5)	2 (3)	4 (4)
Hematological Complications					
Anemia	66 (67)	52 (51)**	25 (66)	33 (52)	60 (61)
Other	40 (40)	40 (40)	15 (39)	21 (33)	44 (45)
Infection Complications					

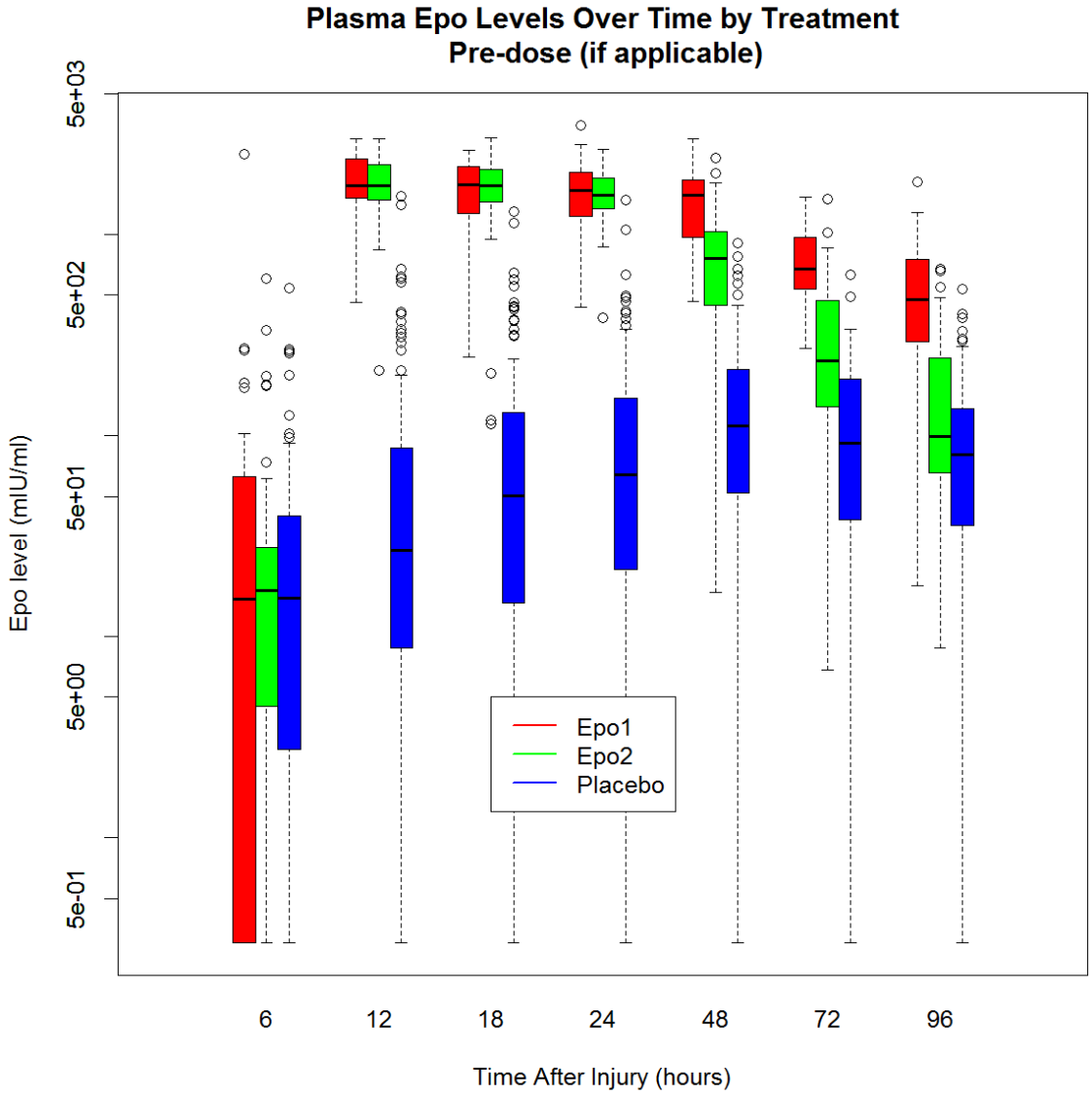
Pneumonia	13 (13)	20 (20)	11 (29)	8 (13)	14 (14)
Urinary tract infection	7 (7)	6 (6)	1 (3)	5 (8)	7 (7)
Meningitis/ventriculitis	3 (3)	3 (3)	2 (5)	2 (3)	2 (2)
Bacteremia	2 (2)	2 (2)	--	1 (2)	3 (3)
Other infections	6 (6)	7 (7)	2 (5)	3 (5)	8 (8)
Sepsis/SIRS/MODS/septic shock	3 (3)	5 (5)	1 (3)	4 (6)	3 (3)
Metabolic Complications					
Diabetes insipidus	8 (8)	4 (4)	1 (3)	2 (3)	9 (9)
Severe hyperglycemia	4 (4)	3 (3)	3 (8)	--	4 (4)
Other metabolic complications	3 (3)	3 (3)	2 (5)	1 (2)	3 (3)
Renal/electrolyte Complications					
Electrolyte disturbances	30 (30)	43 (43)	12 (32)	23 (36)	38 (39)
Acid-base abnormalities	7 (7)	8 (8)	5 (13)	2 (3)	8 (8)
Renal insufficiency/failure	7 (7)	4 (4)	2 (5)	1 (2)	8 (8)
Respiratory Complications					
Atelectasis	21 (21)	27 (27)	14 (37)	14 (22)	20 (20)
ARDS	16 (16)	25 (25)	12 (32)	10 (16)	19 (19)
Pneumothorax	8 (8)	3 (3)	2 (5)	2 (3)	7 (7)
Pleural effusion	5 (5)	4 (4)	--	2 (3)	7 (7)
Airway obstruction requiring re-intubation	4 (4)	1 (1)	1 (3)	3 (5)	1 (1)
Hemothorax	1 (1)	--	--	1 (2)	--
Other Complications	10 (10)	8 (8)	6 (16)	2 (3)	10 (10)

[a] = other cardiovascular complications included superficial thrombophlebitis, gangrene of extremities due to pressors, severe hypertension, and atrial arrhythmia

* = significantly different from placebo group (p<0.01)

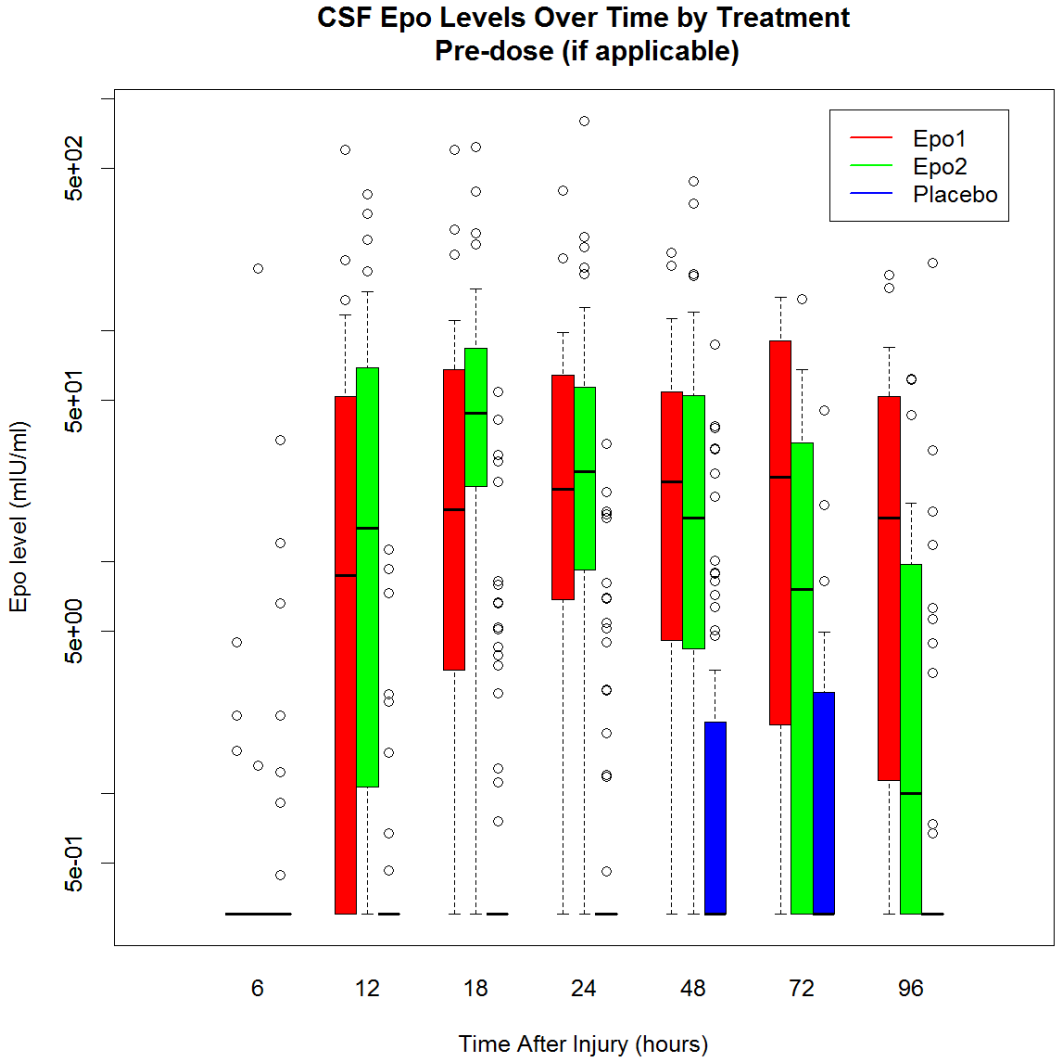
** = significantly different from 7 g/dl transfusion threshold group (p=0·03)

eFigure 1a. Plasma levels of erythropoietin (Epo) over the first four days after injury. The distributions are shown by box plots. The top, bottom, and middle lines of the boxes correspond to the 75th percentile, 25th percentile, and 50th percentile (median), respectively. The whiskers extend to the most extreme data point which is no more than 1.5 times the interquartile range from the box. Observations outside of this range are denoted by a circle.



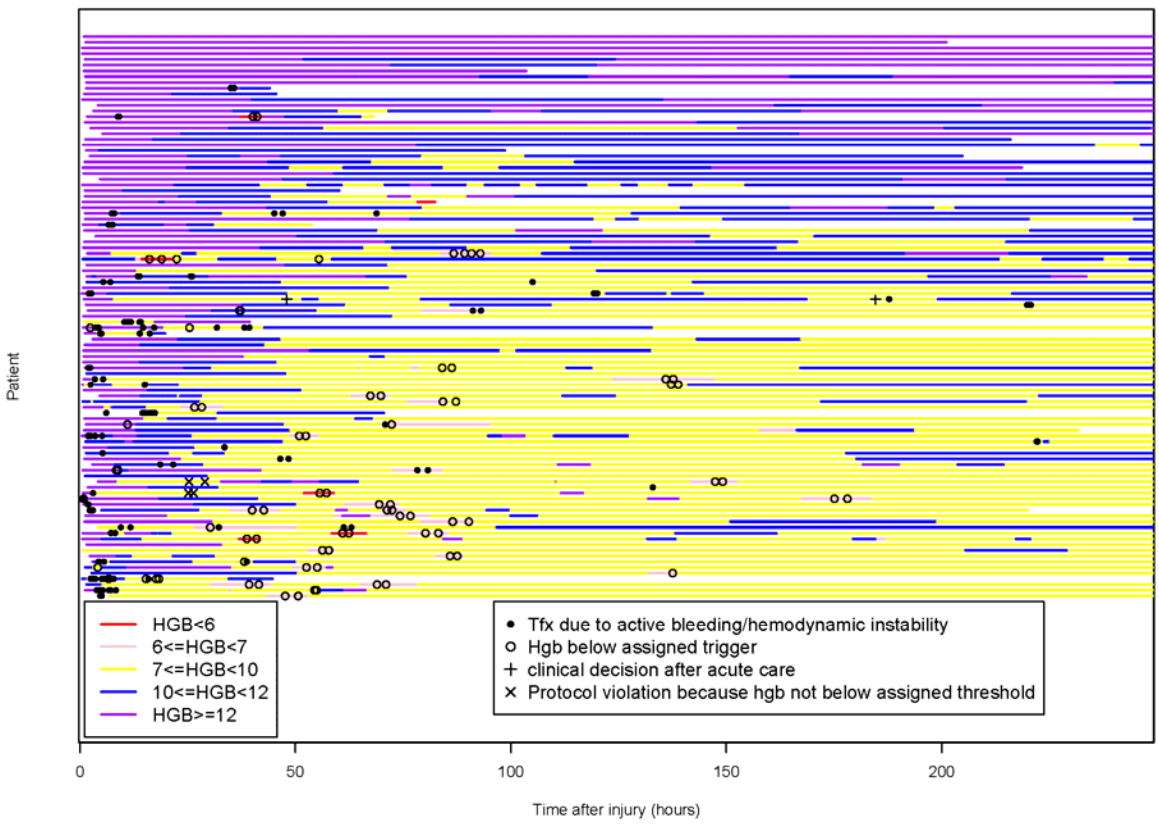
Sample size at each time point for each group	6 Hours	12 Hours	18 Hours	24 Hours	Day2	Day3	Day4
Epo1	32	37	36	37	36	12	29
Epo2	49	60	60	61	57	23	47
Placebo	85	93	92	96	94	23	71

eFigure 1b. CSF levels of erythropoietin (Epo) over the first four days after injury. The distributions are shown by box plots. The top, bottom, and middle lines of the boxes correspond to the 75th percentile, 25th percentile, and 50th percentile (median), respectively. The whiskers extend to the most extreme data point which is no more than 1.5 times the interquartile range from the box. Observations outside of this range are denoted by a circle.



Sample size at each time point for each group	6 Hours	12 Hours	18 Hours	24 Hours	Day2	Day3	Day4
Epo1	17	30	25	27	30	8	22
Epo2	15	47	43	50	45	15	27
Placebo	34	77	80	82	73	20	48

eFigure 2a. Heat maps illustrating the transfusions for individual patients in the 7 g/dl transfusion threshold group. Each horizontal line in the graph is one patient (n=99). The patients are ordered in each graph from top to bottom by decreasing average hemoglobin concentration. The hemoglobin concentration over time is displayed by the color of the line (purple for hemoglobin > 12, blue for hemoglobin 10-11.9, yellow for hemoglobin 7-9.9, pink for hemoglobin 6-6.9, and red for hemoglobin < 6). Each unit of packed red blood cells transfused is indicated by a circle (closed circles when the indication was for active bleeding or hemodynamic instability, and open circles for those units transfused in a hemodynamically stable patient to maintain the assigned hemoglobin level).



eFigure 2b. Heat maps illustrating the transfusions for individual patients in the 10 g/dl transfusion threshold group. Each horizontal line in the graph is one patient (n=101). The patients are ordered in each graph from top to bottom by decreasing average hemoglobin concentration. The hemoglobin concentration over time is displayed by the color of the line (purple for hemoglobin > 12, blue for hemoglobin 10-11.9, yellow for hemoglobin 7-9.9, pink for hemoglobin 6-6.9, and red for hemoglobin < 6). Each unit of packed red blood cells transfused is indicated by a circle (closed circles when the indication was for active bleeding or hemodynamic instability, and open circles for those units transfused in a hemodynamically stable patient to maintain the assigned hemoglobin level).