The Postinjury Inflammatory State and the Bone Marrow Response to Anemia

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Abstract

Rationale: The pathophysiology of persistent injury-associated anemia is incompletely understood, and human data are sparse.

Objectives: To characterize persistent injury-associated anemia among critically ill trauma patients with the hypothesis that severe trauma would be associated with neuroendocrine activation, erythropoietin dysfunction, iron dysregulation, and decreased erythropoiesis.

Methods: A translational prospective observational cohort study comparing severely injured, blunt trauma patients who had operative fixation of a hip or femur fracture ($n = 17$) with elective hip repair patients ($n = 22$). Bone marrow and plasma obtained at the index operation were assessed for circulating catecholamines, systemic inflammation, erythropoietin, iron trafficking pathways, and erythroid progenitor growth. Bone marrow was also obtained from healthy donors from a commercial source ($n = 8$).

Measurements and Main Results: During admission, trauma patients had a median of 625 ml operative blood loss and 5 units of red blood cell transfusions, and Hb decreased

from 10.5 to 9.3 g/dl. Compared with hip repair, trauma patients had higher median plasma norepinephrine (21.9 vs. 8.9 ng/ml) and hepcidin (56.3 vs. 12.2 ng/ml) concentrations (both $P < 0.05$). Bone marrow erythropoietin and erythropoietin receptor expression were significantly increased among patients undergoing hip repair (23% and 14% increases, respectively; both $P < 0.05$), but not in trauma patients (3% and 5% increases, respectively), compared with healthy control subjects. Trauma patients had lower bone marrow transferrin receptor expression than did hip repair patients (57% decrease; $P < 0.05$). Erythroid progenitor growth was decreased in trauma patients (39.0 colonies per plate; $P < 0.05$) compared with those with hip repair (57.0 colonies per plate; $P < 0.05$ compared with healthy control subjects) and healthy control subjects (66.5 colonies per plate).

Conclusions: Severe blunt trauma was associated with neuroendocrine activation, erythropoietin dysfunction, iron dysregulation, erythroid progenitor growth suppression, and persistent injury-associated anemia.

Clinical trial registered with www.clinicaltrials.gov (NCT 02577731).

Keywords: trauma; injury; erythropoiesis; transfusion; critical care

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At a Glance Commentary

Scientific Knowledge on the

Subject: Fifty-five percent of all critically ill trauma patients receive a red blood cell transfusion during their ICU stay; approximately one-half of all transfusions occur five or more days after ICU admission. The pathophysiology of persistent injury-associated anemia is not completely understood, although several factors may contribute. Preclinical studies have implicated postinjury hypercatecholaminemia in the suppression of erythroid progenitor growth.

What This Study Adds to the

Field: This study demonstrates that severe blunt trauma is associated with neuroendocrine activation, erythropoietin dysfunction, iron dysregulation, erythroid progenitor growth suppression, and persistent injury-associated anemia among human trauma patients.

Anemia affects approximately 95% of all patients who remain critically ill for three or more days (1, 2). Management of severe anemia often involves allogeneic red blood cell (RBC) transfusion, which is associated with immune suppression, infectious complications, and increased mortality (3–5). Fifty-five percent of all critically ill trauma patients receive an RBC transfusion during their ICU stay; approximately one-half of all transfusions occur five or more days after ICU admission (6). Although some late RBC transfusions are attributable to operative and phlebotomy blood loss (7), a growing body of evidence suggests that trauma patients are also affected by iron dysregulation and suppression of erythropoiesis (8–10). Iron supplementation and exogenous erythropoietin administration have not reliably increased Hb levels or decreased RBC transfusions (11–13). Therefore, better strategies are needed to promote erythropoiesis among critically ill trauma patients.

The pathophysiology of persistent injury-associated anemia is not completely understood, although several factors may contribute. Our preclinical studies

implicate postinjury hypercatecholaminemia in the suppression of erythroid progenitor growth in a dose-dependent manner that is reversible by chemical sympathectomy with either propranolol or clonidine (14–16). Pathologic blunting of the erythropoietin response has been observed in heterogeneous ICU populations (2, 17, 18), and animal and human data suggest that this phenomenon affects trauma patients as well (10, 19). Hepcidin, an acute-phase reactant that is upregulated by IL-6 (20, 21), may intensify erythropoietin resistance (22) and also inhibit iron mobilization by internalizing its receptor, ferroportin (23), such that iron uptake by erythroid progenitors, mediated by transferrin and TfR (transferrin receptor) (24), is decreased. Trauma patients are also subjected to prolonged and excessive mobilization of hematopoietic progenitors from the bone marrow to injured tissues, a process that may be potentiated by endogenous corticosteroids (25) and G-CSF (granulocyte colony-stimulating factor) (26, 27). G-CSF, CSF1 (colony-stimulating factor 1), and CSF2 also promote granulopoiesis, which may suppress erythropoiesis by competing for common multipotential progenitors or by

directly affecting erythroid maturation pathways (28–31).

The purpose of this translational study was to determine whether severe trauma is associated with neuroendocrine activation, erythropoietin dysfunction, iron dysregulation, suppression of erythropoiesis, and persistent anemia. We have characterized persistent injury-associated anemia among critically ill trauma patients to compare molecular pathways influencing erythropoiesis among trauma patients, elective hip repair patients, and healthy subjects. Some of the results of these studies have been previously reported in the form of an abstract (32).

Methods

Study Population

We performed a prospective observational cohort study comparing three populations: severely injured blunt trauma patients ($n = 17$), elective hip repair patients ($n = 22$), and healthy control subjects ($n = 8$).

These three groups were included with the rationale that bone marrow function occurs along a continuum from healthy subjects to patients undergoing elective hip surgery to patients with severe traumatic injury,

Table 1. Summary of Study Population, Comparing Severely Injured Trauma Patients with Elective Hip Repair Patients

Patient Characteristic*	Severe Trauma ($n = 17$)	Hip Repair ($n = 22$)	P Value
Age, yr	37 (24–51)	65 (57–71)	<0.001
Female, n (%)	8 (47)	15 (68)	0.209
On admission			
Heart rate, beats/min	96 (91–104)	70 (66–78)	<0.001
Systolic blood pressure, mm Hg	99 (81–118)	126 (114–138)	<0.001
Mean arterial pressure, mm Hg	68 (54–77)	90 (74–96)	<0.001
Hb, g/dl	10.5 (9.0–13.4)	13.7 (12.9–14.7)	0.004
Days from admission to surgery	2.0 (1.0–3.0)	0.0 (0.0–0.0)	<0.001
RBC transfusions before surgery	2.0 (0.0–2.5)	0.0 (0.0–0.0)	<0.001
Preoperative Hb, g/dl	8.8 (7.9–10.9)	13.7 (12.9–14.7)	<0.001
Operative blood loss, ml	350 (225–550)	500 (500–600)	0.028
Intraoperative PRBC transfusions	2.0 (0.0–3.0)	0.0 (0.0–0.0)	<0.001
Postoperative Hb, g/dl	9.0 (8.7–10.2)	10.5 (10.1–11.2)	0.004
ICU length of stay, d	5.0 (3.5–7.0)	0.0 (0.0–0.0)	<0.001
Hospital length of stay, d	13.0 (6.5–23.0)	2.0 (1.0–2.0)	<0.001
Operations after index surgery	2.0 (0.5–3.0)	0.0 (0.0–0.0)	<0.001
Operative blood loss, ml	200 (25–415)	0.0 (0.0–0.0)	<0.001
PRBC transfusions after index surgery	1.0 (0.0–3.0)	0.0 (0.0–0.0)	<0.001
Total PRBC transfusions	5.0 (2.0–7.5)	0.0 (0.0–0.0)	<0.001
Total operative blood loss, ml	625 (450–1243)	500 (500–600)	0.256
Discharge Hb, g/dl	9.3 (8.7–9.9)	10.4 (9.9–11.0)	0.002

Definition of abbreviations: PRBC = packed red blood cell; RBC = red blood cell.

*Data are presented as median (interquartile range) unless indicated otherwise.

and that a novel comparison among all three groups would allow for a deeper understanding of the associations among baseline patient characteristics, the degree of physiologic insult and sympathetic nervous system activation, and the resultant bone marrow response. Inclusion criteria for the trauma cohort were as follows: age at least 18 years, injury severity score at least 15 or hemorrhagic shock present on admission to the emergency department, and open reduction and internal fixation of a hip or femur fracture. Hemorrhagic shock was defined as one or more of the following: systolic blood pressure not exceeding 90 mm Hg, mean arterial pressure not exceeding 60 mm Hg, lactate at least 4 mM, or base deficit at least 5 mEq/L. Inclusion criteria for the elective hip fracture cohort were as follows: age at least 18 years and performance of elective hip repair. Exclusion criteria for all cohorts were as follows: transfer from an outside hospital, survival less than 48 hours, osteomyelitis, oncologic administration within 14 days, chronic steroid or immunosuppressant use, previous bone marrow transplant, CD4 count less than 200/mm³, end-stage renal disease, incarceration, or pregnancy. This study was registered at ClinicalTrials.gov (NCT02577731) and approved by the University of Florida Institutional Review Board (IRB201601386). All elective hip repair patients and nonintubated trauma patients gave written informed consent for the research procedures. For those critically ill trauma patients who were not able to provide written informed consent before tissue sampling, written informed consent was obtained from their surrogate decision maker before tissue sampling, and written informed consent was also obtained from the patient if and when they regained decision-making capacity.

Bone Marrow Collection, Processing, and Analysis

Details on the methods for bone marrow collection, processing, and analysis are provided in the online supplement.

Hematopoietic Progenitor Growth

One milliliter of Dulbecco's modified Eagle's medium was added to 500,000 viable bone marrow cells. Ten microliters of a 10% penicillin-streptomycin solution (ATCC) and 3 ml of MethoCult H4034 Optimum (Stemcell Technologies) were

Figure 1. Severe trauma was associated with neuroendocrine activation, bone marrow hypoxia, and systemic inflammation. (A and B) Severely injured trauma patients had elevated plasma norepinephrine (A) and corticosterone (B) levels compared with patients undergoing elective hip repair and healthy control subjects. (C–F) Trauma patients also had elevated bone marrow HIF-1 (hypoxia-inducible factor 1) (C) and HIF-2 (D) expression and higher plasma IL-6 (E) and CRP (C-reactive protein) (F) levels than elective hip repair and healthy subjects (data are illustrated as median values with interquartile range box and 10th and 90th percentile whiskers; healthy control subjects have median value represented by the dashed line and interquartile range represented by the shaded area; * $P < 0.05$ vs. control, ** $P < 0.05$ between groups). BM = bone marrow.

added to the plate, which was cultured at 37°C. Erythroid colony-forming units (CFU-E, late erythroid progenitors) were identified by morphologic characteristics on light microscopy and quantified as colonies per plate 7 days after culture (10). Erythroid blast-forming units (BFU-E, early erythroid progenitors) and granulocyte, erythrocyte, monocyte, megakaryocyte units (GEMM, multipotential hematopoietic progenitors) were identified by morphologic characteristics on light microscopy and counted 14 days after culture. Colonies were counted by an observer blinded to the origin of the samples.

Plasma Collection and Analysis

Detail on the methods for plasma collection and analysis are provided in the online supplement.

Clinical Data

Clinical data regarding patient characteristics, management, and outcomes were collected using the REDCap/RED-I electronic data capture platform (33). Baseline characteristics included age, sex, admission vital signs, and laboratory values. For trauma patients, this included injury severity score, lactic acid, and base deficit values. Management and outcome parameters included estimated blood loss; packed red blood cell transfusions before, during, and after the index operation; serial Hb levels; and length of stay.

Statistical Analysis

The analytic plan followed STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) recommendations for observational cohort studies. Statistical analysis was performed in SPSS version 24 (IBM). Figures were produced with GraphPad Prism version 6.05 (GraphPad Software), illustrated as boxplots with 10th and 90th percentile values. Continuous variables were compared by the Kruskal-Wallis test and reported as median (interquartile range). Discrete variables were compared by Fisher's exact test and reported as *n* (%). Correlations were assessed by Pearson's *r*. The admission base deficit value was missing at random for one trauma patient. Postoperative and discharge Hb values were missing at random for one hip repair patient. Analyses were performed without imputing missing values, $\alpha = 0.05$. Data are presented as median values (interquartile range) or as *n* (%).

Results

Trauma Patients Had Persistent Anemia out of Proportion to Blood Loss and Red Cell Transfusions

Patient characteristics, management parameters, and outcomes are listed in Table 1. Trauma patients were severely injured, with injury severity score 26 (21–34), lactate 3.4 (2.7–5.2) mM, and base

deficit 6.1 (4.3–12.8) mEq/L. Among trauma patients, the median interval between admission and open reduction and internal fixation of the hip or femur was 2 days (1–3). Compared with hip repair patients, trauma patients were younger (37 vs. 65 yr; $P < 0.001$) and had more tachycardia (96 vs. 70 beats/min; $P < 0.001$) and hypotension (mean arterial pressure, 68 vs. 90 mm Hg; $P < 0.001$) on admission. Trauma patients also had lower Hb levels on admission (10.5 vs. 13.7 g/dl; $P = 0.004$). In addition, trauma patients received a median 2 units of packed red blood intraoperatively, compared with no transfusions among hip repair patients ($P < 0.001$). Postoperatively, Hb levels remained lower in trauma patients (9.0 vs. 10.5 g/dl; $P = 0.004$). Hip repair patients were discharged 2 days later with Hb level 10.4 g/dl; trauma patients spent a median of 13.0 days in the hospital and 5 days in the ICU. Trauma cohort Hb levels decreased by 1.2 g/dl during admission in the context of 625 ml total operative blood loss and a median of five total RBC transfusions.

Severe Trauma Was Associated with Neuroendocrine Activation, Bone Marrow Hypoxia, and Systemic Inflammation

Markers of neuroendocrine activation, bone marrow hypoxia, and systemic inflammation are illustrated in Figure 1. At the time of operative fixation,

Figure 2. Severe trauma was associated with blunted bone marrow EPO (erythropoietin) expression. (A–C) Severely injured trauma patients had elevated plasma EPO levels compared with patients undergoing elective hip repair and healthy control subjects (A), but bone marrow EPO (B) and EPO-R (EPO receptor) (C) expression were significantly higher in elective hip repair patients than in severe trauma patients and healthy control subjects (data are illustrated as median values with interquartile range box and 10th and 90th percentile whiskers; healthy control subjects have median value represented by the dashed line and interquartile range represented by the shaded area; * $P < 0.05$ vs. control, ** $P < 0.05$ between groups). BM = bone marrow.

trauma patients had elevated plasma norepinephrine levels (21.9 ng/ml) compared with hip repair patients (8.9 ng/ml; $P = 0.012$) and healthy control subjects (3.5 ng/ml; $P = 0.036$). A similar pattern was observed for plasma corticosterone, which was higher in trauma patients (9.8 ng/ml) than in hip repair patients (4.6 ng/ml; $P = 0.036$) and control subjects (5.1 ng/ml; $P = 0.014$). Relative to healthy control subjects, trauma patients had increased bone marrow HIF-1 (hypoxia-inducible factor 1) (1.8-fold increase; $P = 0.001$) and HIF-2 (9.4-fold increase; $P = 0.006$) expression. Trauma patients also had higher plasma IL-6 (120.4 pg/ml) than hip repair patients (5.2 pg/ml; $P < 0.001$) and healthy control subjects (0.4 pg/ml; $P = 0.024$). Trauma patients had significantly higher plasma C-reactive protein (191.6 ng/ml) than hip repair patients (12.3 ng/ml; $P < 0.001$) and healthy control subjects (1.3 ng/ml; $P < 0.001$). C-reactive protein was also significantly higher in hip repair patients than in healthy control subjects ($P = 0.033$).

Severe Trauma Was Associated with Blunted Bone Marrow Erythropoietin Expression

Erythropoietin and erythropoietin receptor analyses are illustrated in Figure 2. As expected, trauma patients had significantly elevated plasma erythropoietin levels (113.3 mIU/ml) compared with hip repair patients (8.3 mIU/ml; $P < 0.001$) and healthy control subjects (6.4 mIU/ml; $P = 0.036$). Hip repair patients had significantly increased bone marrow erythropoietin expression compared with healthy control subjects (23% increase; $P = 0.001$) and trauma patients (19% increase; $P = 0.005$). Similarly, bone marrow erythropoietin receptor expression among trauma patients was only slightly higher than that of healthy control subjects, and the difference was not statistically significant (5% increase; $P = 0.610$). Hip repair patients had significantly increased bone marrow erythropoietin expression compared with healthy control subjects (14% increase; $P = 0.014$) and trauma patients (9% increase; $P = 0.007$).

Severe Trauma Was Associated with Dysregulated Iron Trafficking

Mediators of iron mobilization and uptake by progenitor cells are illustrated in Figure 3. Trauma patient plasma hepcidin was significantly increased (56.3 ng/ml)

Figure 3. Severe trauma was associated with dysregulated iron trafficking. (A) Severely injured trauma patients had elevated plasma hepcidin levels compared with patients undergoing elective hip repair and healthy control subjects. (B and C) Bone marrow expression levels of ferroportin (B) and transferrin (C) were increased among trauma patients and elective hip repair patients compared with healthy control subjects. (D) Bone marrow TfR-1 (transferrin receptor 1) expression was significantly higher among elective hip repair patients compared with trauma patients (data are illustrated as median values with interquartile range box and 10th and 90th percentile whiskers; healthy control subjects have median value represented by the dashed line and interquartile range represented by the shaded area; * $P < 0.05$ vs. control, ** $P < 0.05$ between groups). BM = bone marrow.

compared with hip repair patients (12.2 ng/ml; $P = 0.002$) and healthy control subjects (13.6 ng/ml; $P = 0.036$). Bone marrow ferroportin expression was increased among trauma (7.6-fold increase; $P = 0.001$) and hip repair patients (4.3-fold increase; $P < 0.001$) relative to healthy control values, without significant differences between trauma and hip repair patients. A similar pattern was observed for bone marrow transferrin expression (trauma: 6.8-fold increase, $P < 0.001$; hip repair: 3.5-fold increase, $P = 0.032$). However, trauma patients had a 0.5-fold decrease in bone marrow TfR-1 expression, whereas hip repair patients had a 1.1-fold increase in TfR-1 expression ($P = 0.012$).

Severe Trauma Was Associated with Myeloid Shift away from Erythropoiesis toward Granulopoiesis

Factors promoting granulopoiesis and hematopoietic progenitor mobilization after an acute physiologic insult are illustrated in Figure 4. Plasma G-CSF was increased among trauma patients (98.8 pg/ml) compared with hip repair patients (32.5 pg/ml; $P = 0.002$) and healthy control subjects (23.7 pg/ml; $P = 0.036$). Both trauma and hip repair patients had increased bone marrow G-CSF expression relative to healthy control subjects (337.4-fold increase, $P < 0.001$; 12.7-fold increase, $P = 0.001$, respectively), and the magnitude of increase was greater among trauma patients ($P = 0.021$). Bone marrow CSF1-R

Figure 4. Severe trauma was associated with a myeloid shift away from erythropoiesis and toward granulopoiesis. (A and B) Severely injured trauma patients had elevated plasma (A) and bone marrow (B) G-CSF (granulocyte colony-stimulating factor) levels compared with patients undergoing elective hip repair and healthy control subjects. (C and D) Trauma patients also had elevated bone marrow expression of CSF1-R (colony-stimulating factor 1 receptor) (C) and CSF2-R (D) compared with elective hip repair and control subjects (data are illustrated as median values with interquartile range box and 10th and 90th percentile whiskers; healthy control subjects have median value represented by the dashed line and interquartile range represented by the shaded area; * $P < 0.05$ vs. control, ** $P < 0.05$ between groups). BM = bone marrow.

(colony-stimulating factor 1 receptor) expression was increased among trauma patients (6.1-fold increase; $P < 0.001$) and hip repair patients (3.4-fold increase; $P < 0.001$), with a proportionately greater increase among trauma patients ($P = 0.010$). A similar pattern was observed for bone marrow CSF2-R expression, with increased expression among trauma patients (6.3-fold increase; $P < 0.001$) and hip repair patients (1.4-fold increase; $P = 0.001$), with a proportionately greater increase among trauma patients ($P < 0.001$).

Severe Trauma Was Associated with Decreased Erythroid Progenitor Growth

Bone marrow cellularity and hematopoietic progenitor colony growth are illustrated in

Figure 5. Bone marrow cellularity was lower in trauma patients (4.7 million cells/ml bone marrow) than in hip repair patients (20.8 million cells/ml bone marrow; $P = 0.010$) and healthy control subjects (16.7 million cells/ml bone marrow; $P = 0.020$). GEMM growth was somewhat decreased among trauma and hip repair patients than in healthy control subjects, but there were no significant differences among groups. Trauma patients had lower BFU-E growth (37.0 colonies per plate) than hip repair patients (54.0 colonies per plate; $P < 0.001$) and healthy control subjects (50.0 colonies per plate; $P = 0.039$). Trauma patients also had significantly lower CFU-E growth (39.0 colonies per plate) than hip repair patients (57.0 colonies per plate; $P = 0.002$) and healthy control subjects (66.5 colonies per plate; $P = 0.004$). Among trauma and hip

repair patients, plasma norepinephrine levels correlated negatively with GEMM growth ($r = -0.508$, $P = 0.045$), BFU-E growth ($r = -0.506$, $P = 0.027$) and CFU-E growth ($r = -0.633$, $P = 0.005$).

Discussion

Critically injured trauma patients developed severe systemic inflammation and neuroendocrine activation that is associated with erythropoietin dysfunction, iron dysregulation, suppression of erythroid progenitor growth, and a clinical phenotype consistent with persistent injury-associated anemia. Hip repair patients exhibited evidence of moderate systemic inflammation, but had upregulation of bone marrow erythropoietin and molecular pathways for iron mobilization and uptake by erythroid progenitors, phenomena that were attenuated or absent in trauma patients. On the basis of these findings and previous work discussed below, we propose a central hypothesis for the pathophysiology of persistent injury-associated anemia, illustrated in Figure 6: neuroendocrine activation and systemic inflammation propagate iron dysregulation, mobilization of hematopoietic progenitors to injured tissues, and suppression of erythroid progenitor growth.

Postinjury hypercatecholaminemia has been implicated in the pathophysiology of persistent injury-associated anemia in preclinical studies. *In vitro*, supraphysiologic catecholamine levels suppress erythroid progenitor growth in a dose-dependent manner that appears to be mediated by bone marrow stromal cells (14, 15). A similar process occurs *in vivo*, and is reversed by chemical sympathectomy (16). In a rodent model of blunt traumatic injury and hemorrhagic shock, the addition of daily restraint stress was associated with persistent elevation of urine norepinephrine levels, decreased erythroid progenitor growth, and anemia that was more severe than in animals subjected to injury and shock alone (19). Although the adrenal medulla is the primary source of the circulating norepinephrine measured in the present study, central sympathetic tone likely contributes via innervation of the adrenal glands and liver and by pre- and postganglionic neurons, promoting hypercatecholaminemia, hypercortisolemia, acute-phase reactants such as hepcidin, and

Figure 5. Severe trauma was associated with decreased erythroid progenitor growth. (A) Severely injured trauma patients had decreased bone marrow cellularity compared with patients undergoing elective hip repair and healthy control subjects. (B) Growth of granulocyte, erythrocyte, monocyte, megakaryocyte (GEMM) colonies was not significantly different among trauma, elective hip repair, and control subjects. (C and D) However, growth of erythroid blast-forming units (BFU-E) (C) and colony-forming units (CFU-E) (D) was significantly decreased among trauma patients compared with elective hip repair and control subjects (data are illustrated as median values with interquartile range box and 10th and 90th percentile whiskers; healthy control subjects have median value represented by the dashed line and interquartile range represented by the shaded area; * $P < 0.05$ vs. control, ** $P < 0.05$ between groups). BM = bone marrow.

inhibiting negative acute-phase reactants such as transferrin.

Blunting of the erythropoietin response has been previously reported in heterogeneous populations of critically ill subjects (2, 17, 18), suggesting a common etiology that may involve systemic inflammation. Persistent neuroendocrine activation may also contribute. In an animal model of blunt traumatic injury and hemorrhagic shock, the addition of daily restraint stress was associated with persistent erythroid progenitor suppression anemia despite significantly elevated erythropoietin levels compared with injury and shock alone (19). Among human trauma patients, plasma erythropoietin is significantly elevated compared with

healthy control subjects, but compensatory reticulocytosis is inadequate, implicating defective erythropoiesis (10), consistent with our findings and others (34, 35). After severe trauma, serum erythropoietin may be low or only mildly elevated, and erythropoietin levels do not appear to correlate well with Hb concentrations (36). However, circulating erythropoietin levels alone may be inadequate for characterizing erythropoietin resistance, considering our observation that bone marrow erythropoietin and EPO-R (erythropoietin receptor) expression were elevated among hip repair patients, but not trauma patients, despite the presence of end-organ hypoxia. Decreased bone marrow EPO-R expression did

correlate with decreased BFU-E and CFU-E growth in trauma patients. In addition to decreased bone marrow erythropoietin and EPO-R, hepcidin may blunt the response to erythropoietin. Among patients on hemodialysis, increased hepcidin levels have been associated with greater erythropoietin resistance, calculated as the ratio of exogenous erythropoietin administration to serum Hb levels (22).

Hepcidin may also contribute to dysregulated iron trafficking after severe trauma. Exogenous hepcidin administration and genetic upregulation of hepcidin have been associated with decreased iron bioavailability and iron-restricted erythropoiesis (37, 38); genetic inactivation of hepcidin has been associated with iron overload (39, 40). After an acute physiologic insult, elevated IL-6 levels stimulate hepcidin production (20, 21). The hepcidin receptor, ferroportin, is present on the surface of enterocytes, macrophages, and hepatocytes. Hepcidin internalizes ferroportin within the host cell, degrading ferroportin and decreasing mobilization of iron stores (23). By this mechanism, hepcidin may reduce the amount of iron available for uptake by erythroid progenitors. Notably, allogeneic red cell transfusion is associated with increased circulating hepcidin levels (41). Therefore, severely injured trauma patients who receive red cell transfusions may be especially vulnerable to hepcidin-mediated iron-restricted anemia. Unexpectedly, we found that trauma patients had increased bone marrow expression of ferroportin despite elevated circulating hepcidin levels. However, iron bioavailability must be accompanied by iron uptake in erythroid progenitors, which is facilitated by transferrin and its receptor via receptor-mediated endocytosis (24). Although transferrin is a negative acute-phase reactant, bone marrow transferrin expression was elevated among trauma and hip repair patients. However, bone marrow TfR-1 expression was decreased, representing the rate-limiting component of iron uptake by erythroid progenitors in the present study. Finally, in response to blood loss and exogenous erythropoietin, erythroblasts produce the hormone erythroferrone, thereby suppressing hepcidin and promoting stress erythropoiesis (42, 43). However, erythroferrone levels decrease after red cell transfusion, which

Figure 6. Central hypothesis for the pathophysiology of persistent injury-associated anemia: Neuroendocrine activation, systemic inflammation, and allogeneic red cell transfusion propagate iron dysregulation, mobilization of hematopoietic progenitors to injured tissues, and suppression of erythroid progenitor growth. CSF1/2-R = colony-stimulating factor 1 receptor and colony-stimulating factor 2 receptor; EPO = erythropoietin; EPO-R = erythropoietin receptor; G-CSF = granulocyte colony-stimulating factor; NE = norepinephrine; TfR = transferrin receptor.

often accompanies severe trauma (43). Future research should assess the impact and relative contributions of blood loss, dysregulated endogenous erythropoietin physiology, and blood transfusion on erythroblast production of erythroferrone among severely injured trauma patients, with attention to temporal relationships between erythroferrone modulators and erythroferrone production.

After severe injury, hematopoietic progenitors mobilize from the bone marrow to the peripheral blood and sites of injury, promoting wound healing and tissue repair, but decreasing the number of progenitor cells available for maturation in the bone

marrow (44, 45). In a rodent lung contusion model, hematopoietic progenitor mobilization was decreased by nonselective β -adrenergic receptor blockade, suggesting that catecholamines may promote progenitor mobilization (46). Neuroendocrine activation may contribute to progenitor mobilization via downstream effects on corticosteroids and colony-stimulating factors. Circulating corticosterone, under the direct influence of the neuroendocrine stress response, has been shown to promote hematopoietic stem cell mobilization (25). In a murine burn sepsis model, chemical sympathectomy abrogated the effects of granulocyte and macrophage colony-stimulating factors in

promoting expansion of hematopoietic progenitors (47). G-CSF is a potent stimulator of hematopoietic progenitor mobilization (26, 27), and has been associated with increased progenitor mobilization after severe trauma (45, 48). In addition to effects on hematopoietic progenitor mobilization, G-CSF potentiates granulopoiesis, which may occur at the expense of erythropoiesis (28, 29). Similarly, upregulation of CSF1 and its receptor promote granulocyte expansion (30, 31). Myeloid shifts away from erythropoiesis have been demonstrated in burn patients (49, 50), and may contribute to anemia in trauma patients as well.

The observation that bone marrow colony growth and cellularity were decreased shortly after trauma is consistent with previous reports of early bone marrow suppression after trauma and other systemic pathophysiologic insults such as sepsis (51). Early upregulation of proapoptotic pathways appears to contribute to this phenomenon (51). Anemia due to early-onset bone marrow failure may be compounded by blood loss due to phlebotomy, increasing the likelihood that critically ill patients will be subjected to allogeneic red cell transfusion and its sequelae (52).

This study was limited by small sample sizes, data from a single institution, and the lack of serial biochemical peripheral blood analyses. Preclinical studies will be useful in establishing causality for persistent injury-associated anemia. Although sample sizes were small, the results suggest that enrollment was adequate. Because results from a single institution may not

be generalizable, external validation is warranted. Future research should perform similar analyses at later time points, and seek to develop therapeutic strategies. Attenuating the neuroendocrine stress response with β -adrenergic receptor blockade promotes erythroblast maturation in animal models of burn injury (49, 50) and trauma (53), with similar effects in severely injured trauma patients (54). Evidence suggests that nonselective β -blockade with propranolol may decrease transfusion requirements among burn patients (55). Hepcidin modulation also requires further investigation. The hepcidin antagonist lexaptide has demonstrated efficacy in increasing serum iron levels in human endotoxemia (56). Critically ill trauma patients are being recruited to a randomized trial of intravenous iron administration with and without oral administration of oxandrolone, a commercially available anabolic steroid

that may mitigate the effects of hepcidin (NCT02047552).

Trauma patients exhibited a proinflammatory state with neuroendocrine activation, erythropoietin dysfunction, iron dysregulation, erythroid progenitor suppression, and persistent anemia. Hip repair patients had compensatory upregulation of the erythropoietin response and molecular pathways for iron mobilization and erythroid progenitor iron uptake, phenomena that were attenuated or absent in trauma patients. Hypercatecholaminemia and hepcidin physiology appear to be central in the pathophysiology of persistent injury-associated anemia. ■

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