

Nutritional Deficiencies and Blunted Erythropoietin Response as Causes of the Anemia of Critical Illness

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Purpose: The purpose of this article was to determine the prevalence of iron, vitamin B₁₂, and folate deficiency and to evaluate the erythropoietin (EPO) response to anemia in a cohort of long-term intensive care unit (ICU) patients.

Materials and Methods: All patients admitted to three academic medical center multidisciplinary ICUs were screened for eligibility into a randomized trial of EPO for the treatment of ICU anemia. On their second or third ICU day, patients enrolled in this trial had EPO levels drawn and were screened for iron, B₁₂, and folate deficiency. Weekly EPO levels were obtained throughout patients' ICU stay.

Results: A total of 184 patients were screened for iron, B₁₂, and folate deficiency. Sixteen patients (9%) were iron deficient by study criteria, 4 (2%) were B₁₂ deficient, and 4 (2%) were folate deficient. Mean hemoglobin and reticulocyte percents of the remaining 160

patients were 10.3 ± 1.2 g/dL and $1.66 \pm 1.09\%$, respectively. In most patients, serum iron and total iron binding capacity levels were very low, whereas ferritin levels were very high. Mean and median day 2 EPO levels were 35.2 ± 35.6 mIU/mL and 22.7 mIU/mL, respectively (normal = 4.2-27.8). Serial EPO levels in most persistently anemic patients remained within the normal range.

Conclusions: In this cohort, screening for iron, B₁₂, and folate deficiency identified potentially correctable abnormalities in more than 13% of patients and should be considered in those who are anticipated to have long ICU stays. Even at an early point of critical illness, most patients had iron studies consistent with anemia of chronic disease (ACD), as well as a blunted EPO response that may contribute to this ACD-like anemia of critical illness.

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ANEMIA, an extremely common disorder in critically ill patients, results in many transfusions in long-term ICU patients.^{1,2} The potential causes of this anemia include blood loss from diagnostic phlebotomy and gastrointestinal (GI) bleeding, decreased erythrocyte life-span due to hemolysis and disseminated intravascular coagulation (DIC), and decreased erythrocyte production or ineffective erythropoiesis secondary to many factors. Multiple investigators have evaluated various simple, effective methods to decrease blood loss from diagnostic phlebotomy, such as the use of in-line blood conservation devices and smaller sampling tubes.³⁻⁷ Likewise, the utility of various measures to prevent gastrointestinal bleeding in ICU patients and the causes of DIC have been extensively addressed.⁸⁻¹⁵ However, the prevalence of nutritional

deficiencies as potentially reversible causes of ineffective erythropoiesis and ICU anemia have been less well described.

In a recent analysis of patients treated in an ICU for more than 3 days, Von Ahsen et al¹⁶ determined that diagnostic phlebotomy accounted for approximately 17% of total blood loss, and overt GI bleeding was present in a few patients. However, most of the progressive anemia they documented could not be attributed to these two causes, and they postulated that a blunted erythropoietin response, decreased iron availability, and nutritional factors may be largely responsible for this anemia. The purpose of this analysis was to characterize factors contributing to ineffective erythropoiesis in a cohort of ICU patients. As part of a trial of recombinant human erythropoietin (EPO) for the treatment of anemia in critically ill patients, we assessed the prevalence of potentially correctable nutritional causes of anemia, specifically iron, B₁₂, and folate deficiency. We also further evaluated the frequency of a blunted EPO response as a contributor to ineffective erythropoiesis in this study population.

MATERIALS AND METHODS

All patients admitted to three academic medical center multidisciplinary ICUs were screened for enrollment into a randomized trial of EPO that is described in detail elsewhere.¹⁷ Dates of enrollment were as follows: Dartmouth-Hitchcock

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Table 1. Inclusion/Exclusion Criteria for Recombinant Human EPO Trial**Inclusion Criteria**

1. Age: 18 years or older.
2. Sex: male or female. Female subjects must have been post-menopausal for at least 1 year or surgically sterile hysterectomy or tubal ligation. Female subjects of childbearing potential must have had a negative pregnancy test (serum hCG RIA) immediately before study entry.
3. No B₁₂ or folate deficiency.
4. No iron deficiency (transferrin saturation <15% and ferritin <50 ng/mL).
5. Hematocrit <38%.
6. Subject (or next of kin) must have read and signed the informed consent form.

Exclusion Criteria

1. Presence of any primary hematologic disease.
2. Risk of hospital death (APACHE II) >80%.
3. Neutropenia (<500 neutrophils) or thrombocytopenia (<20,000 platelet).
4. Vasopressor requirement for blood pressure support (other than low-dose dopamine <5 µg/kg/min) and severe respiratory compromise (Fio₂>60% and/or PEEP >10).
5. Chronic renal failure on maintenance dialysis.
6. Liver failure, cirrhosis, varices, hepatic encephalopathy.
7. Seizures within prior 6 months.
8. Hypertension not controlled on medication (systolic >200, diastolic >110).
9. Severe head injury.
10. Recent neurosurgical procedure or cerebrovascular accident (within 1 month).
11. Recent androgen therapy (within 1 month).
12. Recent cytotoxic or immunosuppressive therapy (within 1 month).
13. Autoimmune hemolysis (Coombs positive).
14. Subjects who have received an experimental drug within 30 days before this study.
15. Previous rHuEPO or involvement in prior rHuEPO clinical study.
16. Subjects prohibited from receiving blood transfusions.
17. Pregnancy or lactation.
18. Active collagen-vascular disease.
19. Recent thromboembolic disease (within 6 months).
20. Active bleeding.

Medical Center (November 1993 to July 1997), Stanford University Medical Center (May 1995 to July 1997), and Naval Medical Center San Diego (November 1995 to July 1997). Inclusion and exclusion criteria for this study are listed in Table 1. At all three hospitals, IRB approval and informed, written consent were obtained. Patients who met entry criteria and gave consent were screened for iron, B₁₂, and folate deficiency on their second or third ICU day. EPO levels were also drawn at this time with serial levels obtained at weekly intervals for as long as the patients remained in the ICU. B₁₂ and folate deficiency were defined by a finding of a serum level below the accepted range of normal at each institution's laboratory. For the purposes of this analysis, iron deficiency was defined as an iron/total iron binding capacity (Fe/TIBC) ratio of <15% combined with a ferritin <100 ng/mL (a higher cut-off value than the above-mentioned exclusion criteria).

Assays for iron studies, serum B₁₂, and folate levels were performed at each hospital's local laboratory. Samples of serum for EPO levels were frozen at -70 degrees Centigrade and sent to a single commercial laboratory (LabCorp, Raritan, NJ) for EPO assay (enzyme immunoassay).

RESULTS

A total of 5,288 patients were admitted to the three ICUs over the study of period. Sixty-six per-

cent of these patients (3,510) either died or were discharged before ICU day 3 and were therefore excluded from screening. Of the remaining, 1,778 patients, 1,594 were excluded on the basis of the listed study criteria, with new onset seizures, primary hematologic disease or malignancy, and liver failure being the most common reasons for ineligibility. Only 1% of patients was excluded because of hematocrit greater than 38%. The remaining 184 patients were screened for iron, B₁₂, and folate deficiency. Sixteen screened patients (9%) were iron deficient by study criteria, 4 (2%) were B₁₂ deficient, and 4 (2%) were folate deficient.

Fifty-four percent of the remaining 160 patients were male. Ages ranged from 18 to 97 years, with a median age of 66 years, and a mean age of 60.3 ± 19.2 years. Their mean, median, and range of length of ICU stay were 18.0 ± 16.5, 12, and 4 to 99 days, respectively. Their three most common admitting diagnoses were pneumonia (24%), other respiratory illness (21%), and post-trauma (15%).

Table 2. Summary of Screening Laboratory Values of Patients Not Iron, B₁₂, or Folate Deficient (N = 160)

	Mean ± SD	Median	Range	Range of Normal	
Hemoglobin (g/dL)	10.3 ± 1.2	10.2	6.9 – 13.1	Male	14–18
Reticulocyte (%)	1.66 ± 1.09	1.3	0.08 – 5.42	Female	12–16
Serum Iron (µg/dL)	275 ± 32.0	17	<2.0 – 276	0.60	1.83
TIBC (µg/dL)	170.2 ± 65.3	163	36 – 378	49	181
Iron/TIBC	0.16 ± 0.18	0.10	0.01 – 0.99	250	450
Ferritin (ng/mL)	726.8 ± 1203.7	384	19 – 10,971	6	>0.15 – 320

Abbreviations: SD, standard deviation; TIBC, total iron binding capacity.

Mean Acute Physiology and Chronic Health Evaluation (APACHE) II score was 18.3 ± 5.4 , and their hospital mortality was 28%. Results of their screening laboratory results are summarized in Table 2. Baseline EPO levels of 113 of these patients are listed in Table 3. Because of incomplete collection of samples, no EPO levels were available for the other 47 (29%) patients. In comparison to the other 113 patients, these patients did not differ significantly with regard to mean age, mean APACHE II scores, mortality, or length of ICU stay. Serial levels of control arm patients (not receiving exogenous EPO) who remained in the ICU are also listed in Table 3.

DISCUSSION

In this study we identified some of the potential causes of ineffective erythropoiesis in a cohort of long-term ICU patients. We identified potentially correctable nutritional causes of anemia in over 13% of patients (9% iron deficiency by our criteria, 2% B₁₂ deficiency, and 2% folate deficiency). In addition, we found that even at an early point of

critical illness (ICU day 2 or 3), most ICU patients had low serum Fe and TIBC, low Fe/TIBC, and elevated ferritin levels—values consistent with ACD. We also documented a blunted EPO response with associated low levels of reticulocytosis in these patients, which is a likely contributor to this ACD-like anemia of critical illness.

Many other investigators have demonstrated the utility of screening less acutely ill populations of anemic patients for iron, B₁₂, and folate deficiency.¹⁸⁻²⁰ Norman and Morrison¹⁸ found a relatively high prevalence of B₁₂ deficiency in the outpatient elderly, and Sumner et al¹⁹ identified evidence of B₁₂ deficiency in approximately 31% of post-gastric surgery patients. Screening for iron deficiency has been even more extensively validated.^{21,22} The prevalence of iron deficiency anemia in the US population is approximately 1% to 2% for adult men and 2% to 5% for adult women,²¹ and screening has been recommended for many patient groups including anemic pregnant patients and anemic infants and children.²²

Fewer investigators have evaluated nutritional screening for anemia in ICU patients. Reductions

Table 3. Initial and Serial Serum EPO Levels (mIU/mL)

	Day 2	Week 1	Week 2	Week 3	Week 4
N	113	41	29	12	9
Mean ± SD	35.2 ± 35.6	28.3 ± 31.5	33.0 ± 54.4	21.5 ± 16.7	29.8 ± 23.6
Median	22.7	15.6	18.0	14.3	26.1
Range	1–166	1–134.6	1–288	1–53	6–84
Percent of values above normal range	40.7	29.3	24.1	25.0	44.4

NOTE. Normal EPO level = 4.2–27.8 mIU/mL.

Abbreviation: SD, standard deviation.

Day 2 column includes levels from 113 of 160 enrolled study patients, whereas week 1, 2, 3, and 4 levels include only those patients who remained in the ICU and did not receive exogenous EPO.

of B₁₂ levels without evidence of true deficiency have been described with prolonged infusions of sodium nitroprusside.²³ During a trial of folate supplementation for ICU patients, Campillo et al²⁴ identified folate deficiency in 19% of studied patients. In other ICU case reports, investigators have characterized the marrow failure that may occur with folate deficiency.²⁵⁻²⁷

A blunted EPO response to anemia has been well documented in chronically ill patients²⁸ and has been cited as a primary cause of the ACD.²⁸⁻²⁹ Several investigators have also examined the EPO response to anemia in critically ill patients.³⁰⁻³⁴ In a study of burn victims, Deitch and Sittig³⁰ found a preserved EPO response to anemia with 82% of severely burned patients producing elevated EPO levels. Conversely, Gabriel et al³¹ and Rogiers et al³² found inappropriately low EPO levels in small cohorts of critically ill adult patients, and Krafte-Jacobs et al³³ demonstrated a decreased EPO response in acutely hypoxic and acutely anemic children. In our large cohort of adult critically ill patients, we confirmed the blunted EPO response to the anemia of critical illness that was observed by these latter investigators. Only 41% of the anemic patients in our study had an elevated day 2 to 3 EPO level, and most of these 41% generated only a marginally elevated response. Even fewer patients (control group) maintained an elevated EPO level over time.

In chronic and critical illness, erythropoiesis is limited not only by blunted EPO production, but also by the suppressive actions of various inflammatory cytokines such as interleukin 1, tumor necrosis factor, granulocyte-macrophage colony-stimulating factor, and gamma-interferon.²⁹⁻³⁵ In an experimental model, immunomodulation with an interferon inhibitor has been shown to reverse some of this erythropoietic suppression.³⁶ A more promising avenue for the treatment of blunted erythropoiesis in the anemia of critical illness lies in the administration of exogenous recombinant human EPO, which has been shown to decrease transfusions in long-term ICU patients.^{17,31}

One of the strengths inherent in the methods of our study was the use of a uniform, early (ICU day 2 or 3) date for screening. This prompt testing may have decreased the influence of potential confounding variables, such as ongoing iron loss from diagnostic phlebotomy and variations in nutritional support during ICU stays. Early screening and di-

agnosis has the additional advantage of maximizing time for intervention to treat these deficiencies.

However, it is possible that the blunted EPO response we found was an artifact of this early screening protocol (insufficient time for the patients to produce EPO), or that we may have missed a peak in EPO production later. However, weeks 2 through 4 EPO levels in the persistently anemic control group (no exogenous EPO) remained low, and other investigators have shown that the normal EPO response to hypoxemia and anemia is rapid and sustained.³⁷ EPO levels of healthy humans increase significantly within 2 hours of acute hypoxic stimulus and remain elevated until removal of this stimulus.³⁷

The heterogeneity of our study population, that is, the mix of multiple principal diagnoses including pneumonia, respiratory failure, postoperative sepsis, and post-trauma, may be considered by some a potential limitation when trying to generalize our findings to more specific groups of ICU patients. Formerly healthy young trauma patients are likely to have a lower prevalence of nutritional deficiencies than older patients with significant prior disease, and they may also generate a more robust EPO response than those with prior renal insufficiency. However, the small numbers within most of these groups, such as trauma or sepsis, precluded meaningful subgroup analysis.

Another important limitation of our work was the relatively large number of study exclusion criteria, which decreased the pool of patients who were screened. A screening protocol that included all long-term ICU patients, not just those who were eligible for the EPO study, would have generated more meaningful results. However, two thirds of ineligible study patients were excluded because they either died or were discharged from the ICU before ICU day 3. Although data regarding these short-term ICU patients may be useful from a public health perspective, they do not represent the group that we were targeting—the long-term ICU patient who is at the greatest risk for further worsening of nutritional deficiencies and anemia.

Perhaps the most significant critiques of this study may lie in the difficulties in defining clinically important nutritional deficiencies and in distinguishing true iron deficiency from ACD in patients with the systemic inflammatory response syndrome (SIRS). Because of variations in distribution of cobalamin (B₁₂) on its binding proteins,

B₁₂ serum levels may not serve as the most clinically useful screens for true deficiency.^{38,39} Recently, developed assays of B₁₂ metabolites may provide more useful information.^{39,40} Given the elevations of the acute phase reactant ferritin that occur in this critically ill population, defining iron deficiency is even more problematic. Most patients had very low iron/TIBC ratios, but none had lower than "normal" ferritin values. This constellation of laboratory values (low Fe/TIBC with normal to high ferritin) makes the distinction between iron deficiency and ACD a difficult one. In a meta-analysis of the literature regarding the laboratory diagnosis of iron-deficiency anemia, Guyatt et al⁴¹ concluded that in the presence of acute inflammation higher than "normal" values of ferritin should be used as cutoff points for iron deficiency. We adopted this recommendation, but it is likely that some of the patients designated as iron deficient by our criteria truly were not iron deficient, and that some patients who had ferritin values >100 ng/mL truly were iron deficient. Measurement of iron stores by bone marrow biopsy is the only true gold standard, but this is impractical to be enacted on a widespread basis in ICUs. Recent work by two groups of investigators may offer better alternatives for the diagnosis of iron deficiency.^{42,43} Herbert et al⁴² found that measurement of iron within ferritin (serum ferritin iron) provides a simple gauge of iron stores that is not confounded by inflammation. Brugnara et al⁴³ have shown that reticulocyte hemoglobin content is the single best screening test for iron deficiency in children.

Finally, in this era of limited health care funding, identification of nutritional causes of anemia must not only be feasible, but also must be cost-effective. In a study of the cost-effectiveness of screening for nutritional causes of anemia in 48 end-stage renal disease patients before EPO therapy, Hutchins and Jones⁴⁴ found that only iron deficiency (38%) was common enough to warrant screening. Their screening protocol identified only one folate deficient patient and no B₁₂ deficient pa-

tients. It could be argued that the 2% yields on screening for these two deficiencies in our patient group also are too low to be cost-effective, and that the poor sensitivity and specificity of macrocytosis for these deficiencies preclude its use in targeting a more selected population for screening.^{45,46} However, identification and treatment of these nutritional deficiencies may provide other clinical benefits, such as the amelioration of neurologic disorders associated with B₁₂ deficiency and the reversal of thrombocytopenia and leukopenia with folate deficiency, in addition to the mere correction of anemia.

Given the relatively high prevalence of iron deficiency and the unavoidable ongoing blood loss in long-term ICU patients, a logical next step would be a study to determine whether providing supplemental iron to these iron deficiency patients truly makes a difference, that is, improves hematopoiesis without significant side effects. As has been demonstrated in patients with rheumatoid arthritis and other chronic inflammatory diseases,⁴⁷ low endogenous EPO levels and cytokine suppression of erythropoiesis in this critically ill group may render iron supplementation without exogenous EPO useless, in which case screening for iron deficiency may be futile.

In this cohort of critically ill patients, screening for B₁₂ and folate deficiency identified correctable abnormalities in 4% of patients and should be considered in those patients who are anticipated to have long ICU stays. Screening for iron deficiency was complicated by the difficulties inherent in defining iron deficiency in patients with acute inflammation. If an iron/TIBC < 15% with a ferritin < 100 ng/mL is accepted as criteria for iron deficiency, then screening may identify iron deficiency in as many as 9% of patients. Most patients in this cohort had iron studies consistent with ACD; they were also found to have a blunted EPO response that may contribute to this ACD-like anemia of critical illness.

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