

## Thrombocytosis in EPO-Treated Dialysis Patients May Be Mediated by EPO Rather Than Iron Deficiency

In a retrospective study of a large cohort of patients with end-stage renal disease (ESRD) published in the October 2008 issue of the *American Journal of Kidney Disease*, Streja et al<sup>1</sup> found relative thrombocytosis (platelet count  $> 300 \times 10^9/L$ ) in 15% of the study population. This was associated with a 30% greater weekly dose of erythropoietin (EPO) and evidence of iron depletion (lower serum transferrin saturation and ferritin concentration). They further found that hemoglobin concentration greater than 13 g/dL ( $>130$  g/L) was associated with heightened mortality in those with, but not those without, relative thrombocytosis. Moreover, the investigators observed a strong correlation between EPO doses of 20,000 U/wk or greater and incidence of relative thrombocytosis, low transferrin saturation, and increased risk of mortality in the study population. They concluded that high doses of EPO can cause relative thrombocytosis by promoting iron depletion in patients with ESRD. In support of this contention, they cited an earlier study that showed an inverse correlation between markers of iron stores and increased platelet counts in a group of women with iron deficiency anemia.<sup>2</sup> Based on these observations, the investigators proposed that the increased mortality observed in the randomized clinical trials of anemia correction<sup>3-5</sup> may have been caused by thrombocytosis caused by iron depletion, occasioned by high doses of EPO. This supposition was endorsed by an editorial review published in the same issue of the journal.<sup>6</sup> The implication of the conclusions drawn by these investigators is that thrombocytosis in this setting can be reversed by iron administration.

The purpose of this communication is to provide evidence that at high doses, EPO can increase platelet production and heighten platelet activity in vivo and in vitro independent of its effect on erythropoiesis or iron metabolism. I believe that clarification of this issue is important, not only from the mechanistic stand point, but also for its potential therapeutic implications, because the notion that thrombocytosis and the associated increase in long-term mortality is caused by reduced iron stores might lead to overzealous use of parenteral iron preparations

and adverse consequences arising from iron overload in the face of prevailing oxidative stress and inflammation in this population.<sup>7,8</sup>

In a prospective, double-blind, randomized, placebo-controlled clinical trial including 244 hemodialysis patients, Kaupke et al<sup>9</sup> showed that administration of a relatively high dose of recombinant EPO (100 U/kg thrice weekly) significantly increases platelet count. They found a significant increase in platelet count within 5 days after the onset of EPO therapy (at which point hemoglobin concentration and hence iron stores were unchanged), reaching a peak at day 40. Moreover, the magnitude of increase in platelet count was similar in patients failing to mount an erythropoietic response and those showing a significant increase in hemoglobin concentration. Finally, the magnitude of the observed increase in platelet count in response to EPO was similar in patients with low serum ferritin and those with normal or high ferritin values. These observations clearly illustrate the dissociation of thrombopoietic effect of EPO from its erythropoietic action and the related impact on tissue iron stores.<sup>9</sup> In confirmation of these findings in patients with ESRD, Stohlawetz et al<sup>10</sup> subsequently showed a significant increase in platelet count in a group of healthy individuals within 3 to 5 days after administration of EPO, at which point hemoglobin concentration and hence iron stores were unchanged.

The independence of the thrombopoietic action of EPO from its impact on iron metabolism and erythropoietic function is supported further by numerous experiments that showed the ability of EPO to stimulate the growth and differentiation of megakaryocytes in vitro.<sup>11-15</sup> Platelets are generated from fragmentation of the cytoplasm of megakaryocytes, which are the byproduct of proliferating

---

Originally published online as doi: 10.1053/j.ajkd.2008.12.030 on March 23, 2009.

Address correspondence to Nosratola D. Vaziri, MD, University of California, Irvine, Division of Nephrology and Hypertension, 101 The City Dr, Bldg 53, Rm 125, Rt 81, Orange, CA 92868. E-mail: ndvaziri@uci.edu

© 2009 by the National Kidney Foundation, Inc.

0272-6386/09/5305-0004\$36.00/0

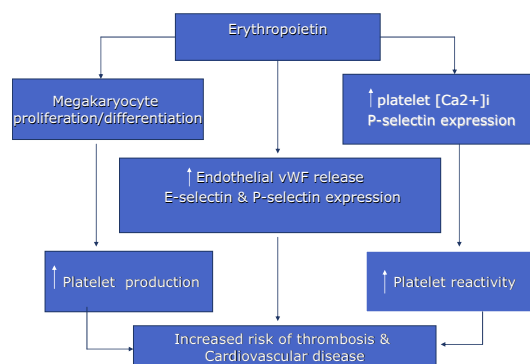
doi:10.1053/j.ajkd.2008.12.030

eration and differentiation of colony-forming unit megakaryocytes (CFU-Megs). CFU-Megs undergo a series of mitotic divisions followed by 3 to 5 synchronized nuclear endomitoses, culminating in formation of large polyploid megakaryocytes. Megakaryocyte volume nearly doubles with each nuclear reduplication cycle. Thus, platelet production capacity depends on both the number and size of megakaryocytes and the balance between the mitotic and endomitotic pathways of megakaryocyte development.<sup>16</sup> Megakaryopoiesis is regulated by a number of hematopoietic growth factors, including interleukin 3 (IL-3), which is a potent stimulant of CFU-Meg proliferation, and IL-6, IL-11, and leukemia inhibitor factor, which predominantly support megakaryocyte maturation.<sup>17</sup> However, EPO enhances both CFU-Meg proliferation and megakaryocyte maturation and platelet production.<sup>14,15</sup> In vitro incubation of cultured bone marrow cells with EPO for 24 to 48 hours in liquid suspension has been shown to increase megakaryocyte colony formation by 50% to 100%.<sup>12</sup> Thrombopoietin, the ligand for the c-mpl receptor, is the major growth factor for megakaryocytic progenitor cells and a key regulator of thrombopoiesis.<sup>18</sup> EPO has been shown to significantly amplify thrombopoietin-induced CFU-Meg proliferation in vitro.<sup>13</sup> Porteu et al<sup>19</sup> have identified functionally important EPO-responsive regions in the cytoplasmic domain of the thrombopoietin receptor, which is required for megakaryocyte differentiation, pointing to the convergence of EPO and thrombopoietin signaling cascades. Interestingly, EPO has been shown to stimulate thrombopoiesis and megakaryocyte proliferation in mice deficient in either thrombopoietin or its receptor in vitro.<sup>20</sup> Clearly, the effects of EPO on megakaryocyte growth and development found in these and other in vitro experiments are independent of iron metabolism. Because megakaryocytes and erythrocytes are derived from a common progenitor, it is not surprising that in addition to its well-known erythropoietic action, EPO has potent thrombopoietic properties.

I want to point out that in addition to increasing platelet count, EPO therapy enhances platelet adhesions and aggregation and shortens bleeding time.<sup>21,22</sup> Moreover, as with platelet production, the effect of EPO on platelet activity is independent of iron stores. This effect is mediated in part by an EPO-induced increase in cytosolic ionized calcium concentration, expansion of intracellular

calcium stores, and intensification of calcium signaling, which is a crucial event in platelet adhesion, aggregation, and contraction and release of platelet-derived mediators.<sup>23</sup> In addition, EPO stimulates expression of P-selectin and E-selectin and production and release of von Willebrand factor in endothelial cells, events that can amplify platelet adhesion and aggregation, respectively.<sup>10,24-26</sup> Thus, by increasing platelet production, augmenting platelet reactivity, and promoting hypertension and vascular remodeling, high doses of EPO can potentially contribute to morbidity and mortality from thromboembolic complications and cardiovascular disease.<sup>27</sup> The effects of EPO on platelet production and activity are shown in Fig 1.

In view of these considerations, the reported association between iron deficiency anemia and thrombocytosis in the general population is caused in part by increased endogenous EPO production as opposed to a direct causal connection. Moreover, the association between relative thrombocytosis and diminished iron stores in patients with ESRD reported by Streja et al<sup>1</sup> represents their surrogacy as opposed to a direct causal relation. Instead, both iron depletion and relative thrombocytosis most likely are driven by high doses of EPO. In this context, the underlying iron deficiency in patients with ESRD necessitates greater doses of EPO in an attempt to achieve the desired hemoglobin level. However, overzealous use of high doses of EPO causes a relative reduction in tissue iron stores (in this case, defined as transferrin saturation < 20%) in



**Figure 1.** Effects of high doses of erythropoietin on platelet production, platelet activity, and potential risks of thromboembolism and cardiovascular disease. Abbreviations: [Ca<sup>2+</sup>]<sub>i</sub>, intracellular ionized calcium concentration; vWF, von Willebrand factor.

an otherwise iron-sufficient patient. In either case, the common denominator is high EPO dosage, which, as shown, can cause thrombocytosis and heightened platelet reactivity. In addition, excessive doses of EPO can cause hypertension and contribute to morbidity and mortality through its extraerythropoietic actions on the cardiovascular system.<sup>27-29</sup> Finally, through the actions of IL-6 and other cytokines, inflammation, which is present to varying degrees in patients with ESRD, can cause reactive thrombocytosis.<sup>30</sup> In addition, inflammation leads to EPO resistance and increased EPO requirement, which can further intensify thrombocytosis. Simultaneously, inflammation results in a significant decrease in serum iron and transferrin values, masquerading as iron deficiency. In view of these considerations, the strong mathematical correlation found between markers of iron depletion and thrombocytosis in the study published by Streja et al<sup>1</sup> reflects circumstantial and indirect association as opposed to direct causal connection and biological relevance.

In summary, I felt compelled to provide this clarification, not only from the mechanistic standpoint, but also for the potential therapeutic implications, because the notion that thrombocytosis and increased long-term mortality in patients with ESRD is caused by iron depletion might lead to overzealous and indiscriminate use of parenteral iron preparations, which can result in adverse consequences arising from iron-mediated amplification of chronic kidney disease-induced oxidative stress and inflammation.

**Nosratola D. Vaziri, MD**  
University of California, Irvine  
Orange, California

#### ACKNOWLEDGEMENTS

*Support:* This work was in part supported by National Institutes of Health Grant 5 U54 RR0119234.

*Financial Disclosure:* None.

#### REFERENCES

1. Streja E, Kovesdy CP, Greenland S: Erythropoietin, iron depletion, and relative thrombocytosis: A possible explanation for hemoglobin-survival paradox in hemodialysis. *Am J Kidney Dis* 52:727-736, 2008
2. Kadikoylu G, Yavasoglu I, Bolaman Z, Senturk T: Platelet parameters in women with iron deficiency anemia. *J Natl Med Assoc* 98:398-402, 2006
3. Besarab A, Bolton WK, Browne JK: The effects of normal as compared with low hematocrit values in patients with cardiac disease who are receiving hemodialysis and epoetin. *N Engl J Med* 339:584-590, 1998
4. Singh AK, Szczech L, Tang KL: Correction of anemia with epoetin alfa in chronic kidney disease. *N Engl J Med* 355:2085-2098, 2006
5. Drueke TB, Locatelli F, Clyne N: Normalization of hemoglobin level in patients with chronic kidney disease and anemia. *N Engl J Med* 355:2071-2084, 2006
6. Littlewood TJ: Normalization of hemoglobin in patients with CKD may cause harm: But what is the mechanism? *Am J Kidney Dis* 52:642-644, 2008
7. Vaziri ND: Oxidative stress in chronic renal failure: The nature, mechanism and consequences. *Semin Nephrol* 24:469-473, 2004
8. Himmelfarb J, Stenvinkel P, Ikizler TA, Hakim R: The elephant in uremia: Oxidant stress as a unifying concept of cardiovascular disease in uremia. *Kidney Int* 62:1524-1538, 2002
9. Kaupke CJ, Butler GC, Vaziri ND: Effect of recombinant human erythropoietin on platelet production in dialysis patients. *J Am Soc Nephrol* 3:1672-1679, 1993
10. Stohlawetz PJ, Dzirlo L, Hergovich N, et al: Effects of erythropoietin on platelet reactivity and thrombopoiesis in humans. *Blood* 95:2983-2989, 2000
11. Ishibashi T, Koziol JA, Burstein SA: Human recombinant erythropoietin promotes differentiation of murine megakaryocytes in vitro. *J Clin Invest* 79:286-289, 1987
12. Dessypris EN, Gleaton JH, Armstrong OL: Effect of human recombinant erythropoietin on human marrow megakaryocyte colony formation in vitro. *Br J Haematol* 65:265-269, 1987
13. Broudy VC, Lin NL, Kaushansky K: Thrombopoietin (c-mpl ligand) acts synergistically with erythropoietin, stem cell factor, and interleukin-11 to enhance murine megakaryocyte colony growth and increases megakaryocyte ploidy in vitro. *Blood* 85:1719-1726, 1995
14. Sakaguchi M, Kawakita M, Matsushita J, Shibuya K, Koishihara Y, Takatsuki K: Human erythropoietin stimulates murine megakaryopoiesis in serum-free culture. *Exp Hematol* 15:1028-1034, 1987
15. An E, Ogata K, Kuriya S, Nomura T: Interleukin 6 and erythropoietin act as direct potentiators and inducers of in vitro cytoplasmic process formation on purified mouse megakaryocytes. *Exp Hematol* 22:149-156, 1994
16. Segal GM, Stueve T, Adamson JW: Analysis of murine megakaryocyte colony size and ploidy: Effects of interleukin-3. *J Cell Physiol* 137:537-544, 1988
17. Debili N, Masse J, Katz A, Guichard J, Breton-Gorius J, Vainchenker W: Effects of recombinant human hematopoietic growth factors interleukin-3, interleukin-6, stem cell factor and leukemia inhibitor factor on megakaryocytic differentiation of CD34+ cells. *Blood* 82:84-95, 1993
18. Kaushansky K, Lok S, Holly RD, et al: Promotion of megakaryocyte progenitor expansion and differentiation by the c-Mpl ligand thrombopoietin. *Nature* 369:568-571, 1994
19. Porteu F, Rouyez MC, Cocault L, et al: Functional regions of the mouse thrombopoietin receptor cytoplasmic

domain: Evidence for a critical region which is involved in differentiation and can be complemented by erythropoietin. *Mol Cell Biol* 16:2473-2482, 1996

20. Carver-Moore K, Broxmeyer HE, Luoh SM, et al: Low levels of erythroid and myeloid progenitors in thrombopoietin- and c-mpl-deficient mice. *Blood* 88:803-808, 1996

21. Cases A, Escobar G, Reverter JC, et al: Recombinant human erythropoietin treatment improves platelet function in uremic patients. *Kidney Int* 42:668-672, 1992

22. Tang WW, Stead RA, Goodkin DA: Effects of epoetin alfa on hemostasis in chronic renal failure. *Am J Nephrol* 18:263-273, 1998

23. Zhou XJ, Vaziri ND: Defective calcium signalling in uremic platelets and its amelioration with long-term erythropoietin therapy. *Nephrol Dial Transplant* 17:992-997, 2002

24. Kahraman S, Yilmaz R, Kirkpantur A, et al: Impact of rHuEPO therapy initiation on soluble adhesion molecule levels in haemodialysis patients. *Nephrology* 10:264-269, 2005

25. Nagai T, Akizawa T, Kohjiro S, et al: rHuEPO enhances the production of plasminogen activator inhibitor-1 in cultured endothelial cells. *Kidney Int* 50:102-107, 1996

26. Borawski J, Naumnik B, Mysliwiec M: Tissue factor and thrombomodulin in hemodialysis patients: Associations with endothelial injury, liver disease, and erythropoietin therapy. *Clin Appl Thromb Hemost* 8:359-367, 2002

27. Vaziri ND: Anemia and anemia correction: Surrogate markers or causes of mortality in chronic kidney disease. *Nat Clin Pract Nephrol* 4:436-445, 2008

28. Vaziri ND: Mechanism of erythropoietin-induced hypertension. *Am J Kidney Dis* 33:821-828, 1999

29. Vaziri ND, Zhou XJ: Potential mechanisms of adverse outcomes in trials of anemia correction with erythropoietin in chronic kidney disease. *Nephrol Dial Transplant* (in press)

30. Kaser A, Brandacher G, Steurer W, et al: Interleukin-6 stimulates thrombopoiesis through thrombopoietin: Role in inflammatory thrombocytosis. *Blood* 98:2720-2725, 2001