

Renal anemia: a nephrologist's view

Tsagalis G

Renal Unit, Alexandra Hospital, Athens, Greece

Abstract

Anemia is a common finding in patients with CKD, with a prevalence that increases gradually as eGFR declines. The prevalence of renal anemia depends on the size of the study and the selection of participants. Diabetic status increases the prevalence of anemia in patients with CKD. Anemia in CKD is due primarily to reduced production of erythropoietin in the kidney and secondarily to shortened red cell survival. Erythropoietin (EPO) is produced by peritubular cells in the kidneys of the adult and in hepatocytes in the fetus. These cells are sensitive to hypoxia that once sensed leads to an increase in EPO production. EPO circulates in the plasma and induces red cell production in the bone marrow after successful binding to erythroid progenitor cells. Apart from EPO, folate, B₁₂ and iron are needed to assure effective erythropoiesis. Factors that can dysregulate this process include inflammation, uremic toxins, hypothyroidism, hypersplenism and ongoing infection.

The investigation of renal anemia requires the assessment of a variety of biological indices. Among them, the complete blood count, the reticulocyte index, B₁₂, folate, ferritin levels and the saturation of transferrin are the most valuable tools in revealing the cause of renal anemia. Hippokratia 2011; 15 (Suppl 1): 39-43

Key words: chronic kidney disease, anemia prevalence, erythropoietin receptors, ferritin, transferrin saturation

Corresponding author: Tsagalis G, 9 Areos str., 15122 Maroussi Attikis, Athens, Greece, Tel : 0030-6942632132, 0030-2108083275, e-mail: tsagalis@otenet.gr

Epidemiology

Anemia is a common finding in patients with CKD, with a prevalence that increases gradually as eGFR declines. Data on the prevalence of renal anemia differ significantly, depending in large part on the size of the study, the selection of participants (general population versus patients already under a physician's care, the definition of anemia and whether they do or do not have diabetes). The National Health and Nutrition and Examination Survey (NHANES) III database was used in 2 different studies that examined the relationship between prevalent Hb concentration and eGFR; their results are consistent with those obtained in ambulatory adult patients. Using a cut-off value of 13g/dl in men and 11g/dl in women, the prevalence of renal anemia increased from 1% at an eGFR of 60ml/min/1,73m² to 9% at an eGFR of 30ml/min/1,73m² and to 33-67% at an eGFR of 15ml/min/1,73m²¹⁻³. However, in NHANES IV, that used a different definition of anemia (World Health Organization criteria: Hb level <12g/dl in women and <13g/dl in men), the results showed a lower prevalence of anemia for each CKD stage⁴. A major limitation of several studies was the relative paucity of data for eGFR levels <30ml/min/1,73m²; the Canadian Multicentre Longitudinal Cohort Study that was designed to address this issue, showed that the prevalence of anemia (defined as an Hb <11g/dl) was greater in the lowest levels of GFR but approached 20% with an eGFR of 30 to 44 ml/min/1,73m²⁵. Diabetic status affects the prevalence of anemia in patients with CKD. In the Kidney Early

Evaluation Program (KEEP 2.0), the prevalence of anemia in diabetic patients was greater than in patients without diabetes at each level of GFR: 8.7% versus 6.9% in stage 2 (p=NS), 7.5% versus 5.0% in stage 3 (p=0.015), 22.2% versus 7.9% in stage 4 (p<0.001) and 52.4 versus 50% in stage 5 (p=0.88)³.

Aetiology-Pathogenesis

Anemia in CKD is due primarily to reduced production of erythropoietin in the kidney (a reflection of reduced renal mass) and secondarily to shortened red cell survival.

Adult humans produce approximately 2.3 million red blood cells every second, or 138 million every minute. The main regulator of that process is erythropoietin, a glycoprotein hormone that circulates at about one hundredth of the concentration of most other hormones in the body^{6,7}. In humans, EPO is produced by peritubular cells in the kidneys of the adult and in hepatocytes in the fetus. These cells (located at the tip of the renal pyramids, susceptible to ischemia), are sensitive to hypoxia that once sensed leads to an increase in EPO production. Although several tissues are able to produce EPO, the main source of EPO is the kidney due to its ability to regulate the hematocrit by matching the plasma volume and the red blood cell (RBC) mass. EPO circulates in the plasma and induces red cell production in the bone marrow⁸, where it binds to erythroid progenitor cells. Cell culture studies have identified two classes of erythroid progenitor cells, BFU-E and colony forming units-erythroid (CFU-E). Both types

of cell have receptors for erythropoietin on their surfaces. When erythropoietin binds to its receptors on BFU-E cells, they proliferate into CFU-E (proerythroblasts). Proerythroblasts are exquisitely sensitive to erythropoietin. They proliferate and develop into erythroblasts and then reticulocytes that enter the peripheral circulation where they mature into red blood cells.

Apart from erythropoietin, several other factors can interfere with erythropoiesis in renal patients. Folate and B₁₂ are needed to assure adequate DNA synthesis especially during the rapid division of erythroblasts. In the absence of iron (blood loss during dialysis, loss from the GI tract, inadequate food intake), the Hb-building steps that follow rapid cell division are affected leading to small, poorly hemoglobinized reticulocytes that emerge from the bone marrow and a hypochromic, microcytic anemia.

Inflammation, a common disorder in CKD, inhibits erythropoietin production, impairs the growth of erythroblasts and promotes death of immature erythroblasts. Inflammation stimulates hepatic release of hepcidin that promotes iron deficient erythropoiesis by both blocking iron absorption in the gut and iron release from resident macrophages.

Uremic toxins, hypothyroidism, hypersplenism and ongoing infection can reduce the erythrocyte life span leading contributing to renal anemia.

EPO production is also influenced by the renin-angiotensin (RAS) system. Angiotensin II causes vasoconstriction of the efferent arteriole, increases GFR and hence the amount of sodium that is filtered. The increase in filtered sodium results in increased oxygen consumption, a drop in oxygen partial pressure that is sensed in the corticomedullary region of the kidney and a subsequent increase in EPO production. Blocking the RAS system abrogates the effect of angiotensin II⁹.

In diabetics, anemia is more prevalent in each stage of GFR. Decreased blood cell survival, impaired absorption of iron and B₁₂, increased tubulointerstitial damage (for each GFR group) and possibly inflammation contribute to renal anemia. The decreased red cell survival induced by uremic toxins is aggravated by the accumulation of advanced glycation endproducts (AGEs) on red cell membrane, while the presence of autoimmune antibodies (20% of patients with type 1 diabetes) against parietal cells leads to autoimmune gastropathy and impaired iron absorption¹⁰. Moreover, antibodies against the intrinsic factor lead to B₁₂ malabsorption¹¹.

Clinical characteristics of renal anemia

As in every case of anemia of chronic disease, the gradual decrease in the circulating red cell mass leads to compensatory responses aiming at reducing hypoxia. Anemia affects every tissue and organ of the human body leading to a great variety of symptoms that were previously attributed to the presence of uremic toxins. The main clinical manifestations of anemia include pallor, cold intolerance, fatigue, anorexia, shortness of breath, reduced libido, tachycardia, irregular menses and angina.

Anemia may aggravate cardiac function through several mechanisms. An increased in cardiac output, left ventricular hypertrophy and angina can compromise cardiac function especially in patients with pre-existing heart failure leading to a vicious circle of cardiorenal insufficiency¹².

Evaluation of the patient with renal anemia

Since erythropoietin is not the only cause of anemia in CKD patients, the initial evaluation should include a variety of tests that provide information about the activity of the bone marrow, the adequacy of iron stores and the availability of iron for erythropoiesis:

- Hb
- Mean corpuscular haemoglobin (MCH)
- Mean corpuscular volume (MCV)
- Mean corpuscular haemoglobin concentration (MCHC)
- White blood cell count (WBC) and differential
- Platelet count
- Absolute reticulocyte count
- Serum ferritin
- Serum transferrin saturation (TSAT) or content of Hb in reticulocytes (CHR)
- B₁₂ and folate blood levels

The initial evaluation of the patients with anemia and CKD should include the analysis of the complete blood count (CBC) that gives valuable information about the severity of anemia, the adequacy of nutrients and the function of bone marrow. Deficiency of folate or vitamin B₁₂ can lead to macrocytosis, whereas iron deficiency or inherited disorders of Hb formation may produce microcytosis. In the case of leukocytosis and thrombocytosis combined with macrocytosis, a generalized disorder of hematopoiesis caused by toxins, nutritional deficit (folate, B₁₂ deficiency), or myelodysplasia should be sought. A hypochromic picture usually reflects long standing iron deficiency anemia.

Typically, the anemia of CKD is normochromic, normocytic and hypoproliferative. Proliferative activity is assessed by determination of the absolute reticulocyte count, the reticulocyte index and the reticulocyte production index:

- The normal reticulocyte count (RC) ranges from 40,000 to 50,000 cells/ μ L of whole blood.
- The reticulocyte index (RI) is calculated from the ratio of observed to normal reticulocyte count.
- The reticulocyte production index (RPI) corrects for the effects of erythropoietin early release from the bone marrow. Upon erythropoietin stimulation, the marrow transit time of reticulocytes shortens while the maturation time in the circulation lengthens. Normal maturation time in circulation is 1 day; the expected maturation time increases to 1.5, 2 and 2.5 days for Hb values between 10-13, 7-10 and <7 g/dl respectively. Therefore, the (RPI) = RI/expected maturation time is a valuable tool (derived from the CBC) that gives information about bone marrow function.

When the severity of anemia, the function of the bone marrow and the adequacy of nutrients (folate, B₁₂) have been assessed, the iron status should be evaluated. Iron status results reflect the level of iron stores or the adequacy of iron for erythropoiesis. The main indices to assess iron stores and iron availability are: serum iron and ferritin levels and transferrin saturation.

Anemia indices in CKD (Fe, Ferritin, TSAT)

Before discussing the role of the main anemia indices in CKD, we will briefly review iron physiology that is fundamental for understanding the terms of functional and absolute iron deficiencies.

Of the approximate 3 grams of body iron in the adult male, approximately 3mg or 0.1% circulates in the plasma as an exchangeable pool. The absorption of iron depends on 3 factors: iron stores, hypoxia and erythropoiesis. Although this issue has not been completely clarified, two models have been proposed in order to explain the regulation of iron absorption: the crypt programming model and the hepcidin model.

1. The crypt programming model: Enterocytes in the crypts of the duodenum take up iron from the plasma. The intracellular iron level of the crypt cells (corresponding to the body's iron stores), determines the amount of iron absorbed from the gut lumen. The crypt cells express both transferrin receptor 1 (TfR1) and TfR2, which mediate the cellular uptake of transferrin-bound iron from plasma¹³⁻¹⁵.

The intracellular iron concentration controls the interaction of cytosolic iron regulatory proteins (IRPs) 1 and 2 with iron regulatory elements in the 3' and 5' regions of different mRNA molecules. In the absence of iron, IRP1 binds to iron responsive elements (IREs) of TfR1 and ferroportin 1 mRNA, the transcript is stabilized, translation proceeds, and the proteins are synthesized. Thus, a high IRP binding activity reflects low body iron stores and results in upregulation of these proteins in the duodenum and increased dietary iron absorption. When IRPs bind to IRE of ferritin mRNA, translation of the transcript is blocked and synthesis is halted. Thus, ferritin levels are regulated reciprocally - being increased in iron-replete states and decreased in iron-deplete states¹³.

2. The hepcidin model: Hepcidin, a 25-amino-acid cysteine-rich peptide produced in the liver is regulated by a number of factors such as liver iron levels, inflammation, hypoxia and anemia. According to this model, hepcidin is secreted into the blood and interacts with villous enterocytes to regulate the rate of iron absorption, by controlling the expression of ferroportin 1 at their basolateral membranes. The binding of hepcidin to ferroportin 1 results in internalization of ferroportin 1 and loss of its function. Ferroportin 1 molecules present in macrophages and liver are also targets for hepcidin. Therefore, whenever hepcidin levels are increased (as in iron overload or inflammation), iron release from intestinal crypt cells, liver and macrophages is reduced. In contrast, when hepcidin levels are reduced, as in iron deficiency, anemia

or hypoxia, it is likely that ferroportin 1 expression and iron release from intestinal cells, liver and cells of reticuloendothelial system is increased¹⁶. In contrast, a mutation in the ferroportin 1 gene is responsible for type IV hemochromatosis.

Essentially all circulating plasma iron normally is bound to transferrin. This chelation serves three purposes: it renders iron soluble under physiologic conditions, it prevents iron-mediated free radical toxicity, and it facilitates transport into cells. Transferrin is the most important physiological source of iron for red cells¹⁷. The liver synthesizes transferrin and secretes it into the plasma. Transferrins are produced locally in the testes and CNS. These two sites are relatively inaccessible to proteins in the general circulation (blood:testis barrier, blood:brain barrier). The locally synthesized transferrin could play a role in iron metabolism in these tissues. Information on the function of transferrin produced in these localized sites is sparse, however. The sum of all iron binding sites on transferrin constitutes the total iron binding capacity (TIBC) of plasma. Under normal circumstances, about one-third of transferrin iron-binding pockets are filled. Consequently, with the exception of iron overload where all the transferrin binding sites are occupied, non-transferrin-bound iron in the circulation is virtually nonexistent.

After binding to its receptor on the cell surface, transferrin is rapidly internalized by invagination of clathrin-coated pits with formation of endocytic vesicles.

Instead of entering lysosomes for degradation, intact receptor-bound apotransferrin recycles to the cell surface, where neutral pH promotes detachment into the circulation¹⁸. Thus transferrin is preserved and reused through pH-dependent changes in the affinity of transferrin for its receptor¹⁹⁻²¹. Iron is further delivered into cells after binding to exported apotransferrin thus each transferrin molecule may be used up to one hundred times for iron delivery²². Within the cell, iron can be stored in two forms: in the cytosol as ferritin and, after breakdown of ferritin within the lysosomes, as hemosiderin. Hemosiderin represents a very small fraction of normal body iron stores, mostly in macrophages, but increases dramatically in iron overload²³. Iron export from macrophages to transferrin is accomplished primarily by ferroportin 1, the same iron-export protein expressed in duodenal enterocytes.

Functional and absolute iron deficiencies are important causes of anemia in CKD, and especially important causes for failure to respond adequately to erythropoietin.

Absolute iron deficiency is likely to be present in patients with end-stage renal disease when either the percent transferrin saturation (plasma iron divided by total iron binding capacity x 100, TSAT) falls below 20 percent or the serum ferritin concentration is less than 100 ng/mL among predialysis and peritoneal dialysis patients or is less than 200 ng/mL among hemodialysis patients. This difference in the serum ferritin level is based upon

accumulating evidence in hemodialysis patients that the maintenance of ferritin levels above 200ng/mL is associated with decreased erythropoietin requirements. True iron deficiency is found in up to 40% of patients with CKD stage 5. However, it is not sufficient for patients with CKD stage 5 to have “normal” iron stores. Patients require high iron availability to maximize use of endogenous erythropoietin and maintain satisfactory hematocrit.

Functional iron deficiency is characterized by the presence of adequate iron stores as defined by conventional criteria, but an inability to sufficiently mobilize this iron from the liver and other storage sites to adequately support erythropoiesis with the administration of erythrocyte stimulating agents (ESA). Typically, these patients have either normal or elevated serum ferritin levels but the transferrin saturation typically is about 20 percent or less.

Inflammatory block is also an important clinical distinction since it usually does not respond to iron. Inflammatory iron block occurs among patients with refractory anemia due largely to an underlying inflammatory state²⁴. However, it should be emphasized that both functional deficiency and inflammatory block may be associated with TSAT \leq 20 percent and ferritin levels between 100 to 800 ng/mL or even higher. The response to ESA and/or parenteral iron may help distinguish between these two possibilities:

In patients with functional deficiency, increasing ESA doses may result in a decrease in ferritin levels while in patients with inflammatory block increased ferritin levels persist, due to persistent inflammation. Moreover, when inflammation is present and the cause is not addressed, the weekly administration of intravenous iron (50 to 125 mg) for up to 8 to 10 doses fails to result in increased erythropoiesis; instead, ferritin concentration progressively rises. By comparison, among patients with functional iron deficiency, additional intravenous iron (in association with an increase in EPO dose) can be effective in increasing Hgb levels, at least over the short-term. This was best shown in the DRIVE study, in which 134 patients with anemia (hemoglobin levels less than 11 g/dL), elevated ferritin levels (500 to 1200ng/mL), low transferrin saturation levels (\leq 25 percent), and high erythropoietin requirements (\geq 225 international units/kg per week or \geq 22,500 international units per week) were randomly assigned to ferric gluconate (125mg with eight consecutive dialysis sessions) or placebo²⁵. Erythropoietin doses were increased in all patients by 25 percent at the beginning of the study. At six weeks, hemoglobin levels had increased significantly more in the active therapy group (1.6 versus 1.1 g/dL). None of the iron parameters typically used in clinical practice, including percent transferrin saturation, ferritin, and reticulocyte hemoglobin content, were found to be particularly sensitive or specific for predicting a response to iron supplementation²⁶. Two of the main concerns of this important study was that some patients who received both intravenous iron and the in-

crease in EPO dose were more likely to have larger, more rapid increases in Hgb level, possibly resulting in adverse effects and that clinical outcomes beyond an increase in Hgb level were not assessed. Nevertheless, the results of the DRIVE study raise the following question: Why nephrologists don't use more iron? A possible answer is that most of us are afraid of iron overload. Although this issue has not been clarified yet, it could well be that this fear is not justified. Feldmann et al showed that high ferritin not cumulative iron dose is linked to increased mortality in hemodialysis patients.

The use of traditional biochemical parameters for the evaluation of anemia in patients with CKD has several limitations. Ferritin, a complex of iron and protein is an acute phase protein; Therefore, ferritin levels do not reflect accurately total iron stores. Serum iron levels fluctuate both during the day and from one day to the other, while transferrin saturation (Fe/total iron binding capacity of transferrin) has a good sensitivity but lacks specificity^{27,28} due to fluctuations in serum transferrin levels. Furthermore the clinical utility of transferrin saturation is impaired by the absence of a diagnostic threshold. Nevertheless, and since the use of newer indices (derived from full blood count) has not yet become routine in every day clinical practice, a combined approach based on iron, ferritin and transferrin saturation (TSAT) can help in the diagnosis of absolute or functional iron depletion:

- Low serum iron and normal or raised iron binding capacity.

- Low serum transferrin saturation (<20%): marker of amount of iron available for incorporation into haemoglobin. % saturation = serum iron/TIBC x 100. Should be maintained >20%.

- Serum ferritin <100 ng/ml: reflects body stores. Also an acute phase protein and increased in inflammation or sepsis. CRP should be measured at the same time. For patients receiving erythropoietin <150 ng/ml inadequate; 150-400 ng/ml probably inadequate (unless haemoglobin well maintained); 400-1000 ng/ml adequate iron stores; >1000 ng/ml iron overload. Increased ferritin is required to restore haemoglobin with increasing anemia.

- Proportion of hypochromic red blood cells >7-10%. Normally <2.5% of red blood cells are hypochromic.

References

1. Astor BC, Muntner P, Levin A, Eustace JA, Coresh J. Association of kidney function with anemia: the Third National Health and Nutrition Examination Survey (1988-1994). *Arch Intern Med* 2002; 162: 1401-1408.
2. Hsu CY, McCulloch CE, Curhan GC. Epidemiology of anemia associated with chronic renal insufficiency among adults in the United States: results from the Third National Health and Nutrition Examination Survey. *J Am Soc Nephrol* 2002; 13: 504-510.
3. El-Achkar TM, Ohmit SE, McCullough PA, et al. Higher prevalence of anemia with diabetes mellitus in moderate kidney insufficiency: The Kidney Early Evaluation Program. *Kidney Int* 2005; 67: 1483-1488.
4. US Renal Data System: USRDS 2004 Annual Data Report. Bethesda, MD, The National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, 2004.

5. Levin A, Thompson CR, Ethier J, et al: Left Ventricular mass index increase in early renal disease: impact of decline in haemoglobin. *Am J Kidney Dis* 1999; 34: 125-134.
6. Lappin TR, Maxwell AP, Johnston PG. EPO's alter ego: erythropoietin has multiple actions. *Stem Cells* 2002; 20: 485-492.
7. Maxwell AP. Novel erythropoiesis-stimulating protein in the management of the anemia of chronic renal failure. *Kidney Int* 2002; 62: 720-729.
8. Erslev AJ, Gabuzda TG. *Pathophysiology of Blood*, third edition. Philadelphia: WB Saunders, 1985: 28-134.
9. Lenga I, Donnelly S. Angiotensin II stimulates erythropoietin production in humans [abstract]. *J Am Soc Nephrol* 2000; 11: 45A. Abstract A0246.
10. Chappay O, Wautier-Pepin MP, Wautier JL. Adhesion of erythrocytes to endothelium in pathological situations: a review article. *Nouv Rev Fr Hematol* 1994; 36: 281-288.
11. Perros P, Singh RK, Ludlam CA, Frier BM. Prevalence of pernicious anaemia in patients with type 1 diabetes mellitus and autoimmune thyroid disease. *Diabet Med* 2000; 17: 749-751.
12. Silverberg D, Wexler D, Iaina A. The importance of anemia and its correction in the management of severe congestive heart failure. *Eur J Heart Fail* 2002; 4: 681-686.
13. Muñoz Gómez M, Campos Garríguez A, García Erce JA, Ramírez Ramírez G. [Fisiopathology of iron metabolism: diagnostic and therapeutic implications]. *Nefrología*. 2005; 25: 9-19
14. Andrews NC. Disorders of iron metabolism. *N Engl J Med* 1999; 341: 1986-1995.
15. Siah CW, Ombiga J, Adams LA, Trinder D, Olynyk JK. Normal iron metabolism and the pathophysiology of iron overload disorders. *Clin Biochem Rev* 2006; 27: 5-16.
16. Nemeth E, Ganz T. Hepcidin and iron-loading anemias. *Haematologica* 2006; 91: 727-732.
17. Ponka P. Tissue-specific regulation of iron metabolism and heme synthesis: distinct control mechanisms in erythroid cells. *Blood* 1997; 89: 1-25.
18. Zak O, Trinder D, Aisen P. Primary receptor-recognition site of human transferrin is in the C-terminal lobe. *J Biol Chem* 1994; 269: 7110-7114.
19. Van Renswoude J, Bridges KR, Harford JB, Klausner RD. Receptor-mediated endocytosis and the uptake of iron in K562 cells: Identification of a non-lysosomal acidic compartment. *Proc Natl Acad Sci USA* 1982; 79: 6186-6190.
20. Klausner RD, van Renswoude J, Ashwell G, Kempf C, Schechter AM, Dean A, Bridges KR. Receptor-mediated endocytosis of transferrin in K562 cells. *J Biol Chem* 1983; 258: 4715-4724.
21. Dautry-Varsat A, Ciechanover A, Lodish HF. pH and the recycling of transferrin during receptor-mediated endocytosis. *PNAS* 1983; 80: 2258-2262.
22. Harford JB, Rouault TA, Huebers HA, Klausner RD. Molecular mechanisms of iron metabolism. In *The Molecular Basis of Blood Diseases*, G. Stamatoyannopoulos, A. W. Nienhuis, P. W. Majerus and H. Varmus, eds. (Philadelphia: W.B. Saunders Co.), 1994; pp. 351-378.
23. Crichton RR, Danielsson BG, Geisser P. Iron metabolism: biologic and molecular aspects. In: Crichton RR, Danielsson BG, Geisser P, editors. *Iron therapy with special emphasis on intravenous administration*. 4th ed. Bremen: UNI-Med Verlag AG; 2008; pp. 14-24.
24. Rambod M, Kovesdy CP, Kalantar-Zadeh K. Combined high serum ferritin and low iron saturation in hemodialysis patients: the role of inflammation. *Clin J Am Soc Nephrol* 2008; 6: 1691-1701.
25. Coyne DW, Kapoian T, Suki W, Singh AK, Moran JE, Dahl NV, Rizkala AR. Ferric Gluconate Is Highly Efficacious in Anemic Hemodialysis Patients with High Serum Ferritin and Low Transferrin Saturation: Results of the Dialysis Patients' Response to IV Iron with Elevated Ferritin (DRIVE) Study. *J Am Soc Nephrol* 2007; 3: 975-984.
26. Singh AK, Coyne DW, Shapiro W, Rizkala AR. Predictors of the response to treatment in anemic hemodialysis patients with high serum ferritin and low transferrin saturation. *Kidney Int* 2007; 11: 1163-1171.
27. Fishbane S, Kowalski EA, Imbriano LJ, Maesaka JK. The evaluation of iron status in hemodialysis patients. *J Am Soc Nephrol* 1996; 7: 265-407.
28. Kalantar-Zadeh K, Hoffken B, Wunsch H, Fink H, Kleiner M, Luft FC. Diagnosis of iron deficiency anemia in renal failure patients during the post-erythropoietin era. *Am J Kidney Dis* 1995; 26: 292-299.