

Iron and erythropoietin in renal disease

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Abstract

Our knowledge of erythropoiesis and iron in renal disease is limited. The accepted view of the control of erythropoiesis was founded on observations made in a variety of disorders, but the control mechanism in healthy individuals may not be quite the same. Evidence suggests that mechanisms other than erythropoietic stimulation may play a role in increased red blood cell production. Measuring erythropoiesis is complex. The quantitative reticulocyte count is probably the closest practical assessment of erythropoietic activity we can achieve, yet there is very little correlation between circulating erythropoietin level and reticulocyte count in normal and near normal subjects. Oxygen transport in humans depends entirely upon iron. In renal disease, the failure of the erythropoietin positive feedback mechanism can be readily and directly remedied; recombinant human erythropoietin therapy can replace the missing erythropoietin, but this will be negated if iron supply to the erythroid marrow falls short of demand. Measurement of iron stores is also complex. The use of serum ferritin concentration as a direct quantitative estimate of iron in the stores is not advisable, and in practice we have not found the transferrin receptor assay to be useful in identifying patients who require iron therapy. Use of percentage hypochromia as a measure of iron deficiency is complicated by the fact that hypochromic cells are not exclusively a consequence of functional iron deficiency. There are clearly lessons still to be learned in this field and there is much that we do not yet understand about the control of erythropoiesis and iron metabolism in humans.

Keywords: anaemia; erythropoiesis; functional iron deficiency; hypochromic red cells; transferrin receptors; transferrin saturation

Introduction

What do we really know about erythropoiesis and iron in renal disease? In 20–30 years time when nephrologists look back on this period, they will realize how little we understood.

We know that there are red blood cells (RBCs) in circulation, that they contain haemoglobin (Hb), and that they carry oxygen. We are aware that healthy subjects produce 2–3 million new RBCs per second but that this rate can rise to 20–30 million RBCs per second. The marrow has enormous expansive properties but does not usually exercise them. Why not? What entity is being regulated? It may be the Hb concentration; it may be the red cell mass; or it may be the rate of oxygen delivery to a particular tissue. Normal RBC production and destruction may even be kept in balance not by how much the marrow is stimulated but what holds it in check and stops RBC production from expanding to 20–30 million per second.

Control of erythropoiesis

The accepted view of the control of erythropoiesis was founded on observations made in a variety of disorders, but the control mechanism in healthy individuals may not be quite the same. Does the body have to become hypoxic to produce erythropoietin? What controls the variation in erythropoietin levels in normal subjects? How does that control erythropoietic production? RBC life span is 120 days on average, but can vary between 75 and 150 days in normal subjects.

Within the normal population there is a 2-fold variation in the rate of erythropoietic activity that is not reflected in the erythropoietin concentration. For example, in early pregnancy RBC production rate increases by a factor of two within the first 12 weeks but erythropoietin concentration does not increase similarly during this time. Erythropoietin concentration increases only late in pregnancy after the RBC expansion has taken place. This suggests that there must be other mechanisms controlling RBC

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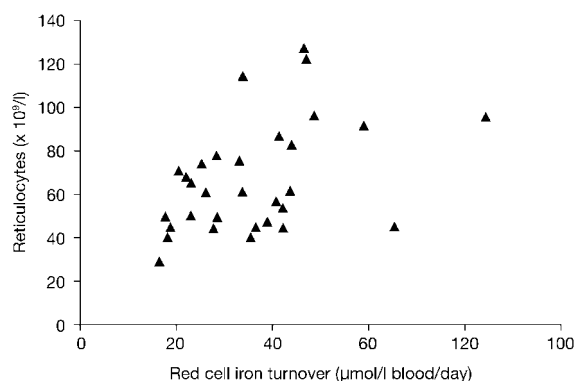


Fig. 1. A very weak relationship was observed between RBC iron turnover and reticulocyte count in patients with renal anaemia receiving continuous ambulatory peritoneal dialysis. Reproduced with kind permission [4].

expansion. In compensated haemolytic anaemias, Hb concentration may be normal yet RBC production is at a level equivalent to, or higher than that seen in patients treated with recombinant human erythropoietin (rHuEPO). Patients with diabetes have a shortened RBC lifespan but they have a matched increase in RBC production that results in normal Hb concentration [1]. Erythropoietin levels are not proportionately raised. This again suggests the involvement of other control mechanisms.

Measures of erythropoiesis

Relatively little is known about oxygen supply in the renal tubules under normal conditions. In the few instances where arterial oxygen saturation and erythropoietin levels have been measured, no clear correlation has been observed between the two. The quantitative reticulocyte count is probably the closest practical assessment of erythropoietic activity we can achieve, yet there is very little, if any, correlation between circulating erythropoietin level and reticulocyte count in normal and near normal subjects. Perhaps because the renal condition, basically erythropoietin deficiency, is considered so straightforward, we have been less aware than we ought to be of the negative feedback factors. The inflammatory cytokines, epitomized by tumour necrosis factor, are likely to have an equally important effect on the net balance of erythropoietic stimulation and inhibition [2].

Considering the parameters that are available as measures of erythropoietic activity, there are limited data on the definitive measure—RBC iron turnover. Investigating RBC iron turnover requires the study of [⁵⁹Fe] clearance from the plasma and its reappearance in RBCs over 2 weeks. Analysis of the data requires considerable methodological, mathematical, and computational resources [3]. This is not a routine clinical investigation. We measured both RBC iron turnover

and reticulocyte count in patients with renal anaemia receiving continuous ambulatory peritoneal dialysis [4]. A very weak relationship was observed between the two (Figure 1). One reason for this may be that the reticulocyte lifespan varies between 0.5 and 2.5 days. This 5-fold proportional variation introduces a previously unsuspected factor into assessing the control of erythropoietic activity by reticulocyte count. However, it is the only parameter that can be routinely used to assess RBC production [5].

Changes in mature RBC lifespan can be inferred from the ratio of reticulocytes to mature cells. This gives the proportion of young cells in circulation. It is informative as a measure of RBC destruction. In the presence of substantially shortened lifespan, reticulocytes will constitute >2.5% of RBCs (Figure 2). Thus, the RBC production rate can be assessed by the reticulocyte count and RBC destruction by reticulocyte percentage.

The role of iron in erythropoiesis

Oxygen transport in humans depends entirely upon iron. This iron must be held in a metastable configuration within a porphyrin ring protected by four globin chains—that is Hb. RBCs are packages of supersaturated Hb. The process of RBC production is highly dynamic. This process normally requires 30–40 mg iron each day to produce some 6 g of new Hb. In renal disease, the failure of the erythropoietin positive feedback mechanism can be readily and directly remedied; rHuEPO therapy can replace the missing erythropoietin, but this will be negated if iron supply to the erythroid marrow falls short of demand.

The majority of the body's iron stored in RBCs is available for recycling at the end of the lifespan of the RBC. When erythropoiesis is suppressed, in particular in chronic inflammatory, infective or malignant conditions, the uptake of recycled iron into the developing RBCs will be limited. In these circumstances, iron released at the end of the lifespan of RBCs will be deposited in the storage iron pool. The major cause of the rise in serum ferritin seen in these conditions is iron being deposited in the reticuloendothelial iron stores, rather than as a putative acute-phase reactant.

The first observation that plasma ferritin levels were related to iron stores was based on 20 local heroes from Cardiff (Figure 3) [6], in whom the readily available iron stores were estimated by quantitative phlebotomy, performed once weekly until a decline in Hb concentration was noted. The amount of iron that had been removed provided a measure of the available iron stores. The data showed that although serum ferritin did reflect iron stores, the relationship was very imprecise.

There is ferritin inside cells and there is ferritin in plasma. Ferritin from both sources has the same protein structure: the same 24 sub-units constitute this

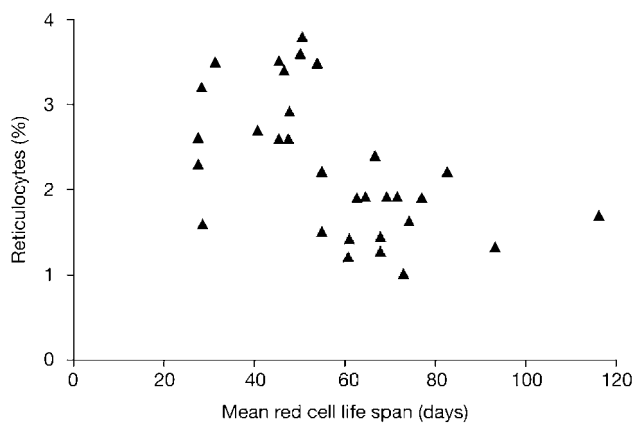


Fig. 2. In the presence of substantially shortened lifespan, reticulocytes will constitute $>2.5\%$ of RBCs.

large 480 000 Da molecule [7]. The protein forms a sphere enclosing a hydrophobic space that is able to contain iron in ferric form (Figure 4). Intracellular ferritin accumulates iron safely in this core. As the core fills, the ferritin molecules become saturated, then coalesce, and eventually form haemosiderin as the protein shell degrades. This insoluble haemosiderin is the stainable iron beloved of microscopists. It does not react with the rest of the biology of the organism. Plasma ferritin is the same protein as that found within the cells but contains no iron. It is secreted from the reticuloendothelial cells: it is not intracellular ferritin that leaks out. The rate of secretion is a function of iron concentration within the cell but the protein that emerges contains virtually no iron. The physiological function of this protein is not known [8].

Because serum ferritin has a lifespan within the circulation of about 3 days, the concentration is the current balance between secretion and clearance. In equilibrium it is indirectly related to iron stores. In disequilibrium, it may be unrelated to iron stores. This was demonstrated by the effect of administration of a single bolus of rHuEPO on the mean plasma ferritin concentration of a group of normal subjects (A. Major 1998, personal communication). In the control group, serum ferritin levels remained constant but, in those who received rHuEPO, serum ferritin dropped from 180 to 100 $\mu\text{g/l}$ over 48 h. If the decrease had been a shift of iron from the stores, it would have represented sufficient iron to make 4 g/dl of Hb. However, in these subjects, Hb concentration remained stable over the 48 h of the study. The large reduction in serum ferritin level was a direct result of shifting iron out of the labile iron pool into developing erythroblasts, stimulated by the erythropoietin. The only incontrovertible statement that can be made about serum ferritin is that if the concentration is less than 12 $\mu\text{g/l}$ there is probably no iron in the stores and if the concentration is more than 20 $\mu\text{g/l}$ there is iron in the stores [9].

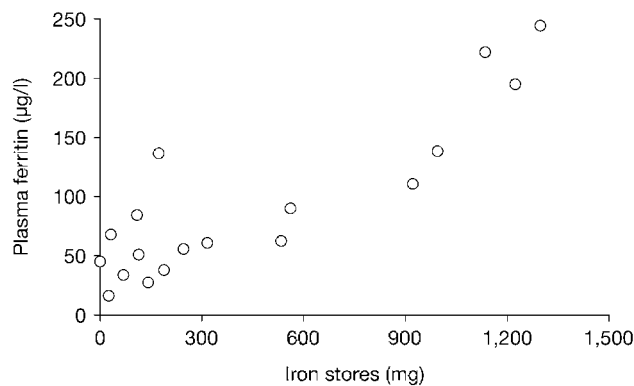


Fig. 3. Correlation of plasma ferritin levels with the amount of iron in the iron stores.

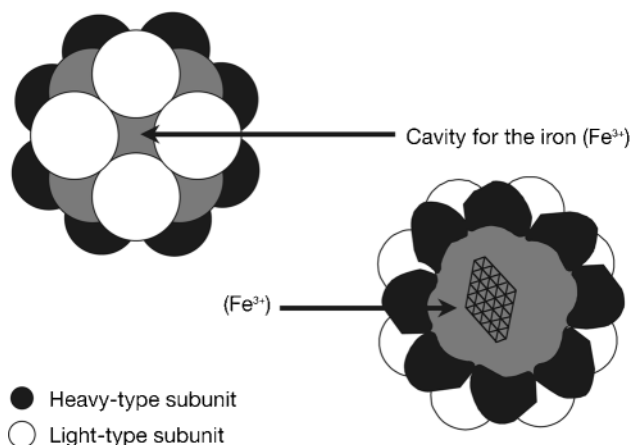


Fig. 4. Structure of the ferritin molecule.

The use of serum ferritin concentration as a direct quantitative estimate of iron in the stores is not advisable. At high levels the measure is misleading because the assays measure both plasma ferritin and released intracellular ferritin. As the concentration of intracellular ferritin is more than an order of magnitude greater than that of plasma ferritin, its release will obscure any variation in plasma ferritin levels.

Hb synthesis

Iron accumulation by erythroblastic cells probably takes place very early in their development. Iron is taken up into the erythroblast ferritin, held there and released for Hb synthesis during the latter part of erythroid development. When the RBC becomes a reticulocyte, uptake of iron and Hb synthesis cease. Erythroblast iron uptake is determined, to a large extent, by the level of transferrin receptors on the cell surface. These receptors bind transferrin-bearing iron on the surface of the cell and then, by a process of endocytosis, take it within the cell. The iron is then

Table 1. Erythropoiesis in rheumatoid arthritis (RA)

	<i>n</i>	Marrow iron turnover ($\mu\text{mol/l}$ blood/day)	RBC iron turnover ($\mu\text{mol/l}$ blood/day)	Ineffective iron turnover (%)	Mean RBC survival (day)
Normal subjects	20	112.7 \pm 27.4	86.1 \pm 19.9	23.1 \pm 6.9	98.0 \pm 22.7
RA iron replete	20	95.8 \pm 55.6	81.4 \pm 50.4	16.1 \pm 4.9	81.3 \pm 36.1
RA iron deficient	12	102.3 \pm 56.8	70.8 \pm 29.9	28.2 \pm 8.0	81.3 \pm 34.0
Simple iron deficiency anaemia	19	94.9 \pm 29.8	64.2 \pm 23.4	32.1 \pm 13.2	80.1 \pm 28.4

Iron kinetic data in normal subjects, iron replete, and iron deficient rheumatoid arthritis with simple iron deficiency anaemia (mean \pm SD).

released and the transferrin receptors recirculate onto the surface of the cell. As the cell develops, the transferrin receptors are lost into the circulation, where their presence can be measured. The assay for transferrin receptor level was first developed as a surrogate marker for the reticulocyte count. Erythropoietic activity includes a considerable degree of intramedullary ineffective erythropoiesis. It has been repeatedly asserted that measuring transferrin receptor level is a means of distinguishing iron deficiency anaemia from the anaemia of chronic disease. However, patients with rheumatoid arthritis demonstrate a wide range of serum transferrin receptor levels (Figure 5) [1]. Often, but not always, serum transferrin receptor levels are low in these patients. The reason for this is related probably to the differing iron kinetics in iron deficiency anaemia and the anaemia of chronic disease. In iron deficient subjects there is a considerable increase in ineffective erythropoiesis (Table 1), causing transferrin receptors to be released into the plasma. In patients with rheumatoid arthritis, ineffective erythropoiesis is decreased. In practice we have not found this assay to be useful in identifying patients who require iron therapy.

Functional iron deficiency results in the production of hypochromic RBCs. However, hypochromic cells are not exclusively a consequence of functional iron deficiency. They result from decreased Hb synthesis whatever its cause. For instance, in patients with thalassaemia unable to produce globin, hypochromia of 5% or more is not an indication of functional iron deficiency. It is still possible to monitor changes in these patients despite a baseline of 20% hypochromic RBCs. In the presence of anaemia of chronic disease, there is growing evidence that the ability to insert iron into the protoporphyrin ring to make haem is itself compromised. This would result in a continuing raised percentage hypochromia. In some circumstances, this has been a good indicator of occult infection. The disadvantage of using percentage hypochromia for this purpose has been that the test is specific to one type of instrument, although this may be about to change.

Predicting benefit of rHuEPO treatment

In patients other than those with renal anaemia, the need for rHuEPO therapy cannot be so readily

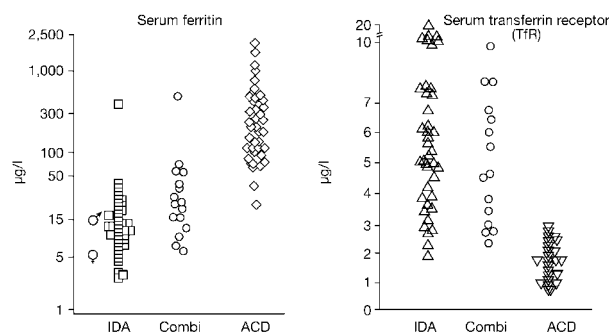


Fig. 5. Serum ferritin and transferrin receptor levels in patients with either iron deficiency anaemia (IDA) or anaemia of chronic disease (ACD) or a combination of both (Combi).

anticipated. In consequence, we have established a service, which aims to assist in predicting the likelihood of a benefit of rHuEPO therapy, by measuring the baseline levels of all erythropoietic parameters. This is not yet a precise science. In diseases such as the myelodysplastic syndrome, patients with extremely high serum erythropoietin levels (700 $\mu\text{g/l}$ or more) often respond to rHuEPO therapy.

Conclusion

There are clearly lessons still to be learned in this field and there is much that we do not yet understand about the control of erythropoiesis and iron metabolism in humans.

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