Is there a role for adjuvant therapy in patients being treated with epoetin?

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Abstract Adjuvant therapy may allow patients being treated with epoetin to derive greater clinical benefits. Iron supplementation is currently the most widely used form of adjuvant therapy; intravenous (i.v.) iron is required by the majority of haemodialysis patients receiving epoetin. Measurement of hypochromic red blood cells is the most direct way of assessing iron supply to the bone marrow. During the correction phase, a dose of i.v. iron equivalent to 50 mg/day is recommended, with the total dose not exceeding 3 g. When subclinical vitamin C deficiency is suspected, ascorbic acid may be given orally (1–1.5 g/week) or i.v. (300 mg three times weekly at the end of dialysis). The active vitamin D metabolite alfalcacidol and calcitriol may, under some circumstances, improve anaemia and reduce epoetin dosage requirements. Vitamin B₆ requirements are increased during epoetin therapy, and supplementation at a dose of 100–150 mg/week is recommended. Supplementation of vitamin B₁₂ is optional. Folic acid is supplemented routinely in haemodialysis patients, though evidence that it increases the efficacy of epoetin is limited. Low doses (2–3 mg/week) should normally be sufficient to maintain optimal folic acid stores in epoetin-treated patients, although higher doses are necessary for patients with hyperhomocysteinaemia. l-Carnitine supplementation may be appropriate in some patients with anaemia of chronic renal failure (CRF) unresponsive to, or requiring large doses of, epoetin. Androgens potentially could reduce epoetin costs in countries with limited resources, but should only be used in men older than 50 years with a remnant kidney. Recent animal studies indicate that the combination of epoetin and insulin-like growth factor 1 might be beneficial in CRF patients. High doses of angiotensin-converting enzyme (ACE) inhibitors should be reserved for dialysis patients who have hypertension that cannot be controlled by other agents, or who require an ACE inhibitor for treatment of heart failure.

Key words: ACE inhibitors; androgens; l-carnitine; cytokines; iron; vitamins

Introduction

The potential role of adjuvant therapies in enhancing the effectiveness of epoetin in chronic renal failure (CRF) patients has received increasing attention in recent years. Adjuvant therapies are important for two reasons. Firstly, they may help to overcome hypo-responsiveness to epoetin, allowing patients to achieve increased haemoglobin concentrations and derive greater clinical benefits. Secondly, they may allow epoetin to be used more cost-effectively.

When haemoglobin does not increase as much as expected in response to epoetin treatment, the presence of either a correctable underlying disorder (such as infection or inflammation) or a deficiency state that could be limiting erythropoesis must be considered. In the last few years, it has become apparent that iron deficiency is the major cause of hyporesponsiveness to epoetin, and most institutions are now fully aware that the majority of haemodialysis patients receiving epoetin will also need intravenous (i.v.) iron supplementation.

Attention has recently turned towards the usefulness of other adjuvant therapies, including vitamins, hormones and cytokines. Nutritional status has always been of concern in patients with CRF; now there is increasing awareness that deficiency states may undermine the effectiveness of epoetin. Although nutritional supplementation has long been routine practice for patients with CRF, recent research highlights the specific roles that some vitamins and l-carnitine may play in the generation of an ongoing supply of healthy red blood cells. The role of cytokines, both stimulatory and inhibitory, is currently under research. In addition, there is some evidence showing that angiotensin-
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converting enzyme (ACE) inhibitors may adversely affect the response to epoetin.

This review summarizes the workshop *Is there a role for adjuvant therapy in patients being treated with epoetin?* and presents the recommendations made by the participants on the basis of the available evidence. The answer to the question posed in the workshop title is certainly ‘yes’; the challenge for the future is to identify which adjuvant therapies offer the greatest clinical and economic benefits for specific patient groups.

### Iron

The response to epoetin is determined primarily by iron availability [1]. In normal circumstances, the major source of iron for erythropoiesis is the iron released from ageing erythrocytes. Any additional iron required is absorbed from the gastrointestinal tract, and iron losses are minimal (0.05% of total body iron). There are no mechanisms (other than blood loss) to excrete excess iron, and any excess is deposited in the reticuloendothelial system as iron stores.

In anaemic patients, however, the amount of circulating iron that is available for new erythropoiesis is diminished and additional iron is required if the haemoglobin concentration is to be increased. For every 1 g/dl increase in the haemoglobin concentration, iron equivalent to a serum ferritin concentration of 20 μg/l is required.

In CRF patients, the supply of iron for the formation of new erythrocytes is reduced, due to both blood loss and reduced absorption. One study has reported that nearly three litres of blood may be lost each year (500 ml in blood taken for laboratory tests and 2400 ml during dialysis procedures) [2]. There may also be additional losses through undiagnosed gastrointestinal bleeding. Absorption of iron via the gastrointestinal mucosa has been shown to be diminished in both haemodialysis and peritoneal dialysis patients [3]. In addition, many patients are treated with drugs that impair iron absorption.

The appropriate use of iron supplementation is, therefore, an important key to successful epoetin therapy. Intravenous iron is more effective than oral iron in increasing erythropoiesis and reducing the epoetin dose required in haemodialysis patients [4], and the i.v. route is now firmly established as the preferred route of iron administration in this patient group [5,6].

Both absolute and functional iron deficiency may be observed in haemodialysis patients receiving epoetin. A condensed algorithm for detecting and managing iron deficiency is shown in Figure 1. Iron management may be divided into three phases: pre-epoetin phase, correction phase and maintenance phase.

#### Pre-epoetin phase

Evidence of absolute iron deficiency should be sought in the pre-epoetin phase. Serum ferritin should be at least 100 μg/l before epoetin is started; a serum ferritin <100 μg/l should be corrected with i.v. iron. If serum ferritin is >200 μg/l at the start of epoetin therapy, no immediate iron supplementation is required, although some nephrologists do continue iron therapy until a serum ferritin of 500 μg/l is reached.

#### Correction phase

Once epoetin therapy has started, functional iron deficiency may occur (i.e. demand may exceed the capacity of the reticuloendothelial stores to release the required amount of iron), even when iron stores are adequate (serum ferritin >200 μg/l). In this situation, there is reduced movement of iron from the diminished labile iron pool to the developing erythroblasts, which gives rise to hypochromic red blood cells (red cells with a haemoglobin concentration <28 g/dl). Hypochromic red cells are a hallmark of functional iron deficiency, and their measurement is the most direct way of assessing whether or not iron supply to the responding marrow is adequate [7]. When the proportion of hypochromic red cells exceeds 10%, i.v. iron is required. A transferrin saturation of <12% also indicates the need for i.v. iron therapy.

Whereas the daily iron requirement for new haemoglobin synthesis is only ~30 mg in normal subjects, erythropoietic activity will typically double with epoetin therapy. The iron deficit will usually be ~50 mg/day. The amount of i.v. iron required should be calculated on this basis, and the total dose given during the correction phase should not exceed 3 g. Levels in excess of 3 g will result in an increasing proportion of the dose being deposited in the iron stores.

Fig. 1. Condensed algorithm for detecting and managing iron deficiency.
Maintenance phase

There is evidence to show that i.v. iron leads to a further stimulation of erythropoiesis and an increase in haemoglobin in iron-replete individuals with a stable haemoglobin and epoetin dose [4,6,8]. Additional iron may therefore allow further bone marrow production, thereby providing extra benefit. The increase in mean corpuscular volume seen with iron therapy in patients with excess iron stores provides indirect evidence for this hypothesis [9].

However, in order to protect patients from possible iron overload, iron therapy should be stopped when the proportion of hypochromic red cells declines to <10%, when transferrin saturation exceeds 40% or when serum ferritin reaches 600–1000 μg/l.

In some continuous ambulatory peritoneal dialysis and pre-dialysis iron-replete patients, who do not have the same degree of blood loss as dialysis patients, oral iron supplementation may be sufficient during the maintenance phase. However, even in these patients, careful monitoring for signs of iron-deficient erythropoiesis (hypochromic red cells, reticulocyte indices) may indicate a need for i.v. iron.

Vitamin C

Haemodialysis may be associated with subclinical vitamin C deficiency, and some nephrologists recommend routine vitamin C supplementation for dialysis patients, whether or not they are receiving epoetin. Oral doses of ascorbic acid are usually in the range of 50–200 mg/day, though some authors recommend higher doses [10].

Recently, it has been shown that i.v. ascorbic acid administration can overcome resistance to epoetin in patients who have functional iron deficiency, even when they are iron overloaded [11,12]. Tarng and Huang treated 12 epoetin-resistant, iron-overloaded patients with ascorbic acid (300 mg i.v. post-dialysis three times weekly) [12]. After 8 weeks of treatment, the haematocrit had increased significantly, with a concomitant significant rise in transferrin saturation, and a decrease in zinc protoporphyrin (ZnPP). Monthly doses of epoetin were also significantly reduced. Possible explanations for this effect of ascorbic acid include increased iron absorption, mobilization of iron from inert tissue stores, and increased iron utilization in the erythron [13].

To determine whether there are any indices that can predict which patients will require i.v. ascorbic acid therapy, Tarng et al. studied 54 iron-overloaded patients (serum ferritin > 500 μg/l) [13]. The treatment group (n = 35) comprised patients hyporesponsive to epoetin, who received ascorbic acid 300 mg three times weekly for 8 weeks. Controls (n = 19) had a haematocrit of >30% and did not receive the adjuvant therapy. Red blood cell and iron metabolism indices and erythrocyte ZnPP were measured before therapy and after 8 weeks. Twelve patients had withdrawn by the end of the study (four control and eight ascorbic acid-treated patients).

The results showed that 13 of the remaining 27 ascorbic acid-treated patients had a good response, with a dramatic rise in haematocrit (25.8±1.7% vs 30.4±2.1%, *P < 0.05*) and a concomitant 20% reduction in epoetin dose after 8 weeks. The enhancement in erythropoiesis paralleled a rise in transferrin saturation, from 28.4±11.2% to 47.9±19%, and a fall in erythrocyte ZnPP, from 123±45 μmol/mol haem to 70±13 μmol/mol haem (*P < 0.05*). In poor responders (14/27) and in controls (n = 15), there were no significant changes in mean epoetin dose, or mean haematocrit, transferrin saturation or ZnPP values.

The 42 patients who completed the study were stratified further into groups of those who did (n = 13) and those who did not (n = 29) require i.v. ascorbic acid. Receiver operator curves showed that two indices predicted response to i.v. ascorbic acid supplementation: erythrocyte ZnPP >90 μmol/mol haem and transferrin saturation <25%. The authors concluded that when epoetin hyporesponsiveness occurs in haemodialysis patients with iron overload, the above values should be used to guide treatment with i.v. ascorbic acid.

Tarng and Huang have also compared the effects of i.v. iron administration with those of i.v. ascorbic acid in 50 epoetin-resistant haemodialysis patients with serum ferritin >500 μg/l [14]. Patients were allocated to one of two protocols: (i) i.v. iron sucrose 100 mg × 5 doses (n = 15) vs controls (n = 11); or (ii) i.v. ascorbic acid 300 mg three times weekly for 8 weeks (n = 12) vs controls (n = 12).

Short-term i.v. iron supplementation in these patients neither improved erythropoiesis nor reduced epoetin doses. In contrast, i.v. ascorbic acid led to a significant increase in haematocrit (Figure 2) and a reduction in the requirement for epoetin. On the basis of the available data, the workshop recommended that when subclinical vitamin C deficiency is suspected, CRF patients not on dialysis should receive oral ascorbic acid 1–1.5 g/day; haemodialysis patients should receive the same regimen, or i.v. ascorbic acid 300 mg three times weekly at the end of dialysis.

There are concerns that, in the long term, high doses of ascorbic acid could lead to oxalate accumulation [15]. However, preliminary data suggest that this potential hazard can be prevented by avoiding vitamin B₆ deficiency [15].

Vitamin D

Secondary hyperparathyroidism (HPT) can aggravate anaemia in patients on haemodialysis. Furthermore, it has been found that the dose of epoetin required to achieve an adequate increase in haemoglobin is related to the severity of secondary HPT and, in particular, to the extent of bone marrow fibrosis [16].

Before the advent of epoetin, it was shown that
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patients was 811 pg/ml at entry, indicating significant secondary HPT. Patients were treated with calcitriol (2 μg i.v. after dialysis, adjusted according to PTH, serum calcium and phosphate levels). After 12 months of treatment, 19 patients were classified as responders, i.e. PTH had decreased. These patients showed a significant increase in mean haemoglobin from 10.6 ± 1.5 g/dl to 12.2 ± 1.5 g/dl (P < 0.001). The increase in haemoglobin in response to calcitriol was correlated with a significant decrease in PTH.

These findings raise an interesting question: does the effect of active vitamin D metabolites on haemoglobin reflect solely the correction of HPT, or could there also be direct effects on erythroid precursor cells in the bone marrow? The hypothesis that there may be direct effects is supported by two findings. Firstly, calcitriol can induce the proliferation and maturation of stem cells in vitro [23]. Secondly, calcitriol treatment in vivo can improve anaemia in some patients, even if it does not lower PTH, without alterations to epoetin dosage [21,24,25]. Further evidence is needed, however, before vitamin D metabolites can be recommended as adjuvant treatments in patients without evidence of HPT.

Vitamin B<sub>6</sub>, vitamin B<sub>12</sub> and folic acid

Deficiencies in vitamin B<sub>12</sub> and folic acid are rare in pre-dialysis patients [26]. However, in both peritoneal dialysis and haemodialysis patients, folic acid and vitamins B<sub>6</sub> and B<sub>12</sub> can be lost in the dialysate, especially during high-flux dialysis. This is because these vitamins are water-soluble and of small-to-medium molecular size [27]. Supplementation of folic acid and vitamins B<sub>6</sub> and B<sub>12</sub> is therefore current practice in many dialysis units, and usually results in normal or higher than normal plasma values.

Vitamin B<sub>6</sub>

Vitamin B<sub>6</sub> is involved in haem synthesis and in the incorporation of iron into haem, and is important as a co-factor in erythropoiesis. Severe vitamin B<sub>6</sub> deficiency may occur in patients with CRF as a result of restricted diet, impaired metabolism of the active form (pyridoxyl-5-pyrophosphate) and losses in either the urine (from frusemide administration) or the dialysate. However, studies of vitamin B<sub>6</sub> status in patients with CRF have yielded contradictory results, probably due to methodological discrepancies.

Recently, a vitamin B<sub>6</sub> deficiency in erythrocytes has been reported in both peritoneal dialysis patients and haemodialysis patients receiving epoetin, despite normal plasma concentrations [28]. The study data suggest that vitamin B<sub>6</sub> requirements are increased in patients receiving epoetin therapy and that a vitamin B<sub>6</sub> deficiency may lead to resistance to the hormone. Vitamin B<sub>6</sub> supplementation at a dose of 100–150 mg/week is therefore considered advisable.
Vitamin $B_{12}$

Vitamin $B_{12}$ deficiency has been reported only once [29]; nonetheless, there may be a long-term risk of deficiency due to losses in the dialysate, especially with high-flux membranes. If and when vitamin $B_{12}$ deficiency occurs, it is likely to be a late event, as the liver stores contain enough of the vitamin to last for 3–5 years. Supplementation of vitamin $B_{12}$ may be regarded as optional; when it is given, a dose of 0.25 mg/month should be sufficient. Tielemans et al. (unpublished data) found that, with i.v. supplementation of vitamin $B_{12}$, 0.5 mg/month, patients on high-flux dialysis maintained a greater than normal plasma concentration throughout a 5 year follow-up period.

Folic acid

Folic acid supplementation has been advocated on the basis of a single 30-year-old publication [30]. However, as today’s diets are less restricted, losses of folic acid in the dialysate have less impact. Consequently, the need for routine supplementation in both epoetin- and non-epoetin-treated patients is now being questioned, [31]. Nonetheless, there is some evidence that folic acid demand may be increased in epoetin-treated patients. Pronai et al. [32] have reported the development of hyporesponsiveness to epoetin therapy, together with macrocytic anaemia, despite initially normal plasma folic acid levels. Response to epoetin increased and mean cell volume decreased in eight patients given folic acid supplementation (10 mg/day).

Low doses (2–3 mg/week) should normally be sufficient to maintain optimal folic acid stores in epoetin-treated patients, although higher doses are necessary for patients with hyperhomocysteinaemia. However, the workshop participants noted that high doses of folic acid (15 mg/day) may increase the risk of thromboembolic events [33]. When assessing folic acid stores, erythrocyte folic acid levels are preferable to serum levels.

Preliminary findings by Sunder-Plassmann et al. (unpublished) suggest that high-dose folate therapy has no beneficial effect on epoetin responsiveness in CRF patients with hyperhomocysteinaemia, despite the finding that elevated total homocysteine concentrations in end-stage renal failure patients can be partially corrected by folic acid.

$\text{L-}-\text{Carnitine}$

Although inadequate secretion of erythropoietin is the critical defect in the anaemia of CRF, shortened erythrocyte survival may also be important. In uraemia, erythrocyte survival time may be reduced due to increased erythrocyte ATP (secondary to decreased sodium/potassium ATPase activity and stimulated glycolysis), alterations in lipid and phospholipid membrane components and impairment of the pentose-phosphate shunt [34].

$L$-Carnitine deficiency is believed to be a key factor underlying some of these processes. L-Carnitine is an important carrier molecule in the transport of long-chain fatty acids across the inner mitochondrial membrane for beta-oxidation, and it appears to be important for membrane phospholipid metabolism. In addition, L-carnitine is responsible for regulating the concentration of co-enzyme A (CoA) and for removing the toxic acyl-CoAs that accumulate in CRF patients due to incomplete oxidation. Deficiency of $L$-carnitine leads to accumulation of toxic acyl-CoAs and low erythrocyte membrane sodium/potassium ATPase activity.

$L$-Carnitine is dialysable, and is depleted from the muscle stores during long-term haemodialysis. Patients on dialysis tend to have reduced free plasma carnitine and markedly elevated plasma acyl carnitine (AC), implying a reduced needs for routine supplementation in both epoetin- and non-epoetin-treated patients. Erythrocyte survival may also be important. In uraemia, erythrocyte survival time may be reduced due to increased erythrocyte ATP (secondary to decreased sodium/potassium ATPase activity and stimulated glycolysis), alterations in lipid and phospholipid membrane components and impairment of the pentose-phosphate shunt [34].

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and endogenous erythropoietin secretion before and after l-carnitine supplementation [36]. However, a study by Matsumura et al. confirmed a correlation between epiotin dosage and free carnitine concentrations, and demonstrated increased red blood cell globular osmotic fragility in patients with a low l-carnitine level [40]. Administration of l-carnitine has also been shown to reduce erythrocyte membrane fragility in dialysed patients [37].

In summary, there is some evidence to support the notion that administration of l-carnitine to haemodialysis patients may improve anaemia and, in turn, reduce epiotin requirements. However, placebo-controlled studies that carefully control for other parameters influencing erythropoiesis are needed before definitive conclusions can be drawn.

A recent consensus group statement [41] concluded that l-carnitine supplementation in dialysis patients should not be routine. This strategy should be reserved only for patients with certain conditions, including the anaemia of CRF unresponsive to, or requiring large doses of, epiotin. Patients with elevated PTH or C-reactive protein (CRP), as well as those with aluminium toxicity or megaloblastosis, should not be treated with l-carnitine. As there are currently only limited data in this area, a pragmatic approach should be adopted. A trial supplementation period may be indicated to exclude l-carnitine deficiency as the cause of epiotin resistance or unresponsiveness in selected patients.

**Androgens**

Before the introduction of epiotin, studies had already been conducted on the efficacy of androgens in treating the anaemia of CRF. Androgens are believed to improve erythropoiesis either via their ability to increase endogenous erythropoietin production from the remnant kidney or by enhancing the sensitivity of erythroid progenitors to available erythropoietin [42].

Since the introduction of epiotin, several studies have examined the potential role of androgens in enhancing response and reducing dose requirements. One 16 week study in 12 men and five women found that intramuscular (i.m.) nandrolone decanoate 2 mg/kg/week plus i.v. epiotin 120 IU/kg/week had no greater effect than epiotin alone [43]. Two patients receiving nandrolone decanoate in this study were withdrawn because of acne.

However, two other studies have shown a significantly greater increase in haematocrit with i.m. nandrolone decanoate than with epiotin alone. In a study by Ballal et al., 15 adult male haemodialysis patients were given epiotin 2000 IU three times weekly, with or without the addition of 100 mg nandrolone decanoate i.m. each week [42]. After 12 weeks of therapy, there was a slight increase in haematocrit in the group receiving epiotin alone (from 25.3% at baseline to 27.4%), but a much more marked increase in haematocrit in the group receiving combination therapy (from 24.4% at baseline to 32.9%, \( P < 0.001 \) vs epiotin alone). No side effects of nandrolone decanoate were noted during this short-term study.

In a longer-term study by Gaughan et al. in 11 men and eight women on haemodialysis, patients received epiotin 1500 IU three times weekly for 6 months, with or without the addition of nandrolone decanoate, 100 mg i.m. weekly [44]. At the end of the study, both groups showed a significant increase in mean haematocrit compared with baseline, but the increase in the androgen-treated group was significantly greater than in the group receiving epiotin alone (8.2% vs 3.5%, \( P = 0.012 \), Figure 3) [44]. No side effects of nandrolone decanoate were noted in this study, except for pain at the injection site.

Other studies, by Teruel et al., have examined the erythropoietic effect of androgen therapy given alone in patients with CRF [45–47]. In the largest of these studies, which included 67 men and 17 women, there was a significant age-related erythropoietic effect of i.m. nandrolone decanoate (200 mg weekly for 6 months). Treatment was associated with an increase in dry weight and serum albumin, a reversible increase in serum triglycerides, and a decrease in lipoprotein(a); there was no change in prostate tumour markers or blood pressure. Most women receiving nandrolone decanoate experienced mild hirsutism and voice change. The authors noted that these side effects were significant disadvantages to treatment.

Even in men, there is concern about negative effects of androgens on the liver and prostate. In the study described above, Teruel et al. found elevations in liver enzymes in three patients with antibodies to hepatitis C, which returned to normal when androgen treatment was stopped. However, none of the 13 patients with biochemical data indicative of liver disease experienced a worsening of the disease during treatment [47]. In another study of 14 men receiving nandrolone decanoate 200 mg i.m. weekly for 6 months, androgen admin-
istration did not induce increases in prostatic tumour markers [45]. Nevertheless, at present, it seems prudent to recommend that the use of androgens as an adjunctive therapy to epoetin be restricted to men older than 50 years with a remnant kidney.

It is possible that androgens could be useful in combination with epoetin in countries where resources are limited. The workshop recommended that a prospective, randomized study be conducted in both predialysis and dialysis patients to compare low-dose s.c. epoetin with low-dose epoetin plus nandrolone decanoate in order to assess whether adjunctive therapy with androgens is cost-effective.

**Cytokines**

Cytokines may either promote or inhibit erythropoiesis. There may be a role for stimulatory cytokines as adjuvant therapy with epoetin, while detection of inhibitory cytokines may help to assess the reasons for hyporesponsiveness to epoetin.

**IGF-1**

Insulin-like growth factor 1 (IGF-1) was first described in 1982 as a new candidate for the regulation of erythropoiesis [48]. IGF-1 receptors are found on erythrocyte precursors and mature erythrocytes, and it is thought that growth hormone can stimulate erythropoiesis via IGF-1.

This ‘somatomedin hypothesis’ is supported by a study of hypophysectomized rats [49], which demonstrated that IGF-1 can stimulate erythropoiesis both directly and indirectly. Growth arrest in hypophysectomized rats not only results in low levels of IGF-1, but also in reduced erythropoiesis. Administration of IGF-1 led to an increase in the rate of erythropoiesis and an increase in serum erythropoietin. However, it was found that the stimulatory effect on erythropoiesis occurred before the increase in serum erythropoietin. Thus, IGF-1 may have both an endocrine and a paracrine mechanism of action. The role played by locally produced IGF-binding proteins has yet to be elucidated, however.

At the cellular level, it is known that both IGF-1 and erythropoietin decrease apoptosis of human erythroid colony-forming cells [50]. Erythropoietin also maintains erythroid cell viability and development, whereas IGF-1 enhances erythroid maturation and proliferation. However, the proliferation of erythroid progenitors is controlled mainly by stem cell factor (SCF) and erythropoietin, independent of an effect on apoptosis. Interferon-γ down-regulates the number of SCF and erythropoietin receptors on erythroid colony-forming cells, but not IGF-1 receptors [51].

In rats, there is evidence that IGF-1 and not erythropoietin modulates erythropoiesis during accelerated growth [52]. It is interesting to note that erythropoiesis is impaired in adult patients with growth hormone deficiency. In a study of growth hormone-deficient adults treated with recombinant human growth hormone (rhGH), there was an increase in erythropoiesis, together with an increase in total blood volume and total body water [53]. It was shown that rhGH had increased serum IGF-1 but not serum erythropoietin.

It was therefore hypothesized that growth hormone and IGF-1 may have direct effects on erythroid and myeloid progenitor precursor cells. In children of short stature who were treated with rhGH, blood haemoglobin was shown to correlate positively with relative body height, serum IGF-1 and IGF-binding protein-3 but not with serum erythropoietin [54]. Similarly, a study in uremic patients with severe HPT has shown that haematocrit correlates better with serum IGF-1 than with serum erythropoietin [55].

Recent unpublished data have also shown that anaemia in dialysis patients is modulated by changes in their IGF-1-binding protein concentrations. Furthermore, the erythrocytosis that occurs following renal transplantation has been found to be due to an abnormality of IGF-1 and its binding proteins [56].

These findings led to the suggestion that IGF-1 might be useful as an adjuvant to epoetin in the treatment of the anaemia of CRF. In a murine model, mice with surgically induced renal failure were treated for 3 weeks with subtherapeutic doses of epoetin, IGF-1, or the combination. Neither agent alone caused any significant change in haemoglobin [57]. However, the combination produced a striking increase in haemoglobin, resulting in correction of anaemia in the majority of animals (Figure 4) [57]. The response to combination therapy was comparable with that seen with the maximal dose of epoetin in a dose-finding study. These data suggest that the combination of epoetin and IGF-1 might be beneficial in CRF patients.
and human studies are now required to test this hypothesis.

**IL-3**

Various other cytokines are thought to enhance erythroid cell proliferation and maturation, including interleukin-3 (IL-3), IL-4, IL-9, IL-11, SCF and granulocyte–macrophage colony-stimulating factor (GM-CSF). It has been suggested that a decrease in stimulatory cytokines, such as IL-3, is a cause of epoetin resistance [58].

In erythropoiesis, IL-3 is an early active cytokine, and IL-3 and GM-CSF receptors share a common beta-chain. Both cytokines enhance erythropoietin-dependent erythropoiesis in vitro by primary haematopoietic progenitor and factor-dependent cells. There is evidence that the beta-chain functionally and physically associates with the erythropoietin receptor, suggesting that these cytokine receptors exist as a large supercomplex. This offers the first molecular explanation for the synergistic effects of IL-3 and GM-CSF with erythropoietin during erythropoiesis.

IL-3 has been shown to increase the number of colonies derived from burst-forming units-erythroid (BFU-E) and colony-forming units-granulocyte macrophage (CFU-GM) grown in the presence of erythropoietin in vitro [59]. One in vitro study took BFU-E from patients with renal anaemia 2 weeks after the start of epoetin therapy. An increase in growth of BFU-E was seen when IL-3 was added to the culture medium, indicating an enhanced sensitivity of BFU-E to IL-3 [60]. Another in vitro study in renal patients demonstrated that the addition of erythroid differentiation factor, either alone or in combination with IL-3, improved a reduced sensitivity of BFU-E to epoetin [61]. Finally, an analysis of circulating haematopoietic progenitors in haemodialysis patients showed that sensitivity of peripheral blood BFU-E to IL-3 was lower in patients with a poor response to epoetin treatment [62].

Many studies have investigated the potential of IL-3 to treat different states of bone marrow failure and haematological malignancies, and to expand haematopoietic progenitor cells for transplantation. However, no clinical use for IL-3 has been established to date [63].

**Inhibitory cytokines**

Recently, Bárány et al. have reported that increased serum CRP is a strong predictor of resistance to epoetin, suggesting increases in inflammatory cytokines (Figure 5) [64]. Epoetin dosage and serum CRP were both inversely correlated with serum albumin and serum iron. These data suggest that the principal mechanism by which inflammatory cytokines inhibit erythropoiesis is linked to iron metabolism (i.e. functional iron deficiency). CRP testing must be included among routine blood tests in haemodialysis patients, assisting the early detection and treatment of epoetin resistance.

ACE inhibitors and response to epoetin

Anaemia sometimes occurs as a side effect of ACE inhibitors given to treat hypertension [65] or heart failure. Studies of patients with renal insufficiency [66] and renal transplant patients [67] also indicate that the anaemia of CRF is aggravated by ACE inhibitors. In 27 enalapril-treated patients with CRF, Kamper et al. [66] found that haemoglobin declined over 90 days from a median of 7.6 mmol/l to 6.7 mmol/l; in a control group of 32 patients haemoglobin remained constant at 7.6 mmol/l (P < 0.001 enalapril vs control). Moreover, in the enalapril-treated group, the median erythropoietin concentration declined from 32 to 24 IU/l, whereas it remained unchanged in control patients (P < 0.05 enalapril vs control).

Vlahakos et al. reviewed 27 transplant patients treated with enalapril and found that 37% had developed an otherwise unexplained anaemia [67]. Julian et al. have also reported that administration of ACE inhibitors to post-transplant patients may suppress erythropoiesis [68].

There is evidence linking the rate of secretion of endogenous erythropoietin in haemodialysis patients to activation of the renin–angiotensin system. When this system is inhibited, erythropoietin secretion decreases. In one study in haemodialysis patients, captopril (50 mg) given prior to a dialysis session completely blocked the expected increase in serum erythropoietin [69].

ACE inhibitors have also been found to markedly increase the plasma natural stem cell regulator N-acetyl-seryl-aspartyl-lysyl-proline (Ac-SDKP). An increase in Ac-SDKP may cause anaemia by preventing the recruitment of pluripotent haematopoietic stem cells and normal early progenitors into S-phase [70].

A recent study by Morrone et al. [71] suggests that
Intravenous iron therapy is required by most haemodialysis patients receiving epoetin. Measurement of hypochromic red cells is the most direct way of assessing whether or not iron supply to the bone marrow is adequate. When the proportion of hypochromic red cells exceeds 10%, i.v. iron is required.

An iron dose equivalent to 50 mg/day is appropriate during the correction phase, although the total dose should not exceed 3 g.

Oral iron supplementation may be appropriate for pre-dialysis patients and those receiving peritoneal dialysis, though i.v. iron may be used if oral iron is not effective.

Fig. 6. Comparison of epoetin requirements in 40 hypertensive patients treated with enalapril (5–20 mg/day) or nifedipine (20–40 mg/day) and in 20 normotensive controls. To maintain a minimum haemoglobin concentration of 10 g/dl, patients receiving enalapril required significantly higher doses of epoetin. (Reproduced with permission from [74]).

IGF-1 plays a role in the ACE inhibitor-related decrease in haematocrit in patients with post-transplant erythrocytosis (PTE). Serum erythropoietin and IGF-1 were significantly greater in patients with PTE than in patients without PTE. Therapy with ACE inhibitors significantly reduced haematocrit, IGF-1 and serum erythropoietin. There was a direct relationship between haematocrit and serum IGF-1, but not between haematocrit and serum erythropoietin. No significant changes in IL-2, IL-3 and GM-CSF were detected.

In some studies, patients treated with ACE inhibitors required a significantly higher epoetin dose to maintain their haemoglobin than those not receiving ACE inhibitors [72–74]. However, other studies have found no such effect [75–78]. One possible explanation for these conflicting findings is that the dose of both epoetin and the ACE inhibitor is important. It may be that a reduced response to epoetin is seen only when the ACE inhibitor is given at a high dose and epoetin at a relatively low dose. When the opposite is true, no effect may be seen.

In a prospective, non-randomized, 12 month study, Albitar et al. compared epoetin requirements in 40 hypertensive patients treated with enalapril (5–20 mg/day) or nifedipine (20–40 mg/day) and 20 normotensive controls [74]. A mean haemoglobin concentration of >10 g/dl was maintained in all groups. The mean weekly epoetin dose increased in the enalapril group (P < 0.0001 vs baseline) and remained constant in the nifedipine and control groups (Figure 6) [74]. The authors of this study concluded that high-dose enalapril increased epoetin requirements. This implies that high-dose ACE inhibitors should be reserved for dialysis patients whose hypertension cannot be controlled by other agents, or patients who require an ACE inhibitor for treatment of heart failure.

**Conclusions**

In summary, the conclusions of this workshop are as follows.

- Intravenous iron therapy is required by most haemodialysis patients receiving epoetin.
- Measurement of hypochromic red cells is the most direct way of assessing whether or not iron supply to the bone marrow is adequate. When the proportion of hypochromic red cells exceeds 10%, i.v. iron is required.
- An iron dose equivalent to 50 mg/day is appropriate during the correction phase, although the total dose should not exceed 3 g.
- Oral iron supplementation may be appropriate for pre-dialysis patients and those receiving peritoneal dialysis, though i.v. iron may be used if oral iron is not effective.
- When subclinical vitamin C deficiency is suspected, CRF patients not on dialysis should receive oral ascorbic acid 1–1.5 g/week; haemodialysis patients should receive the same regimen, or i.v. ascorbic acid 300 mg three times weekly at the end of dialysis. Preliminary data suggest that oxalate accumulation can be prevented by avoiding vitamin B6 deficiency [15].
- Treatment of secondary HPT with the active vitamin D metabolites alfalcacidol or calcitriol improves anaemia. There is also evidence suggesting that these agents may reduce epoetin dosage requirements.
- Vitamin B12 requirements are increased in patients receiving epoetin therapy, and supplementation at a dose of 100–150 mg/week is therefore recommended.
- Supplementation of vitamin B12 is optional; when it is given, a dose of 0.25 mg/month should be sufficient.
- Folic acid demand may be increased by epoetin treatment, though the evidence for the efficacy of supplementation is equivocal. Low doses (2–3 mg/week) should normally be sufficient, although higher doses are necessary for patients with hyperhomocysteinaemia.
- l-Carnitine supplementation may be appropriate in CRF patients on dialysis if their anaemia is unresponsive to, or requires large doses of, epoetin. A trial of l-carnitine may be appropriate in such patients; however, patients with elevated PTH or CRP, as well as those with aluminium toxicity or megaloblastosis, should be excluded.
- Androgens in combination with epoetin could be useful in countries where resources are limited. However, androgen use should be confined to men older than 50 years with a remnant kidney.
- Recent animal studies strongly suggest that the combination of epoetin and IGF-1 might be beneficial in CRF patients. Human studies are now required to test this hypothesis.
- High serum CRP indicates high levels of inflammatory cytokines, and is a strong predictor of resistance to epoetin. In future, routine testing for CRP could assist in the early detection and treatment of epoetin resistance.
- High doses of ACE inhibitors should be reserved
A role for adjuvant therapy in patients treated with epoetin

References


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