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Special Feature

Poor response to erythropoietin: practical guidelines on investigation and management

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Introduction

It is now nearly six years since recombinant human erythropoietin was licensed for the treatment of renal anaemia, and numerous clinical trials worldwide are currently investigating its use in other (non-renal) anaemic conditions. Experience has shown that 90–95% of patients with renal anaemia will respond to erythropoietin [1], although there is a small group who show either no response or a blunted response [2,3]. This minority group comprising 5–10% of patients treated is nevertheless important not only because of the lack of therapeutic efficacy but because they may require or even waste large amounts of this expensive therapy. At current prices in the UK, a 70 kg man failing to respond to a dose of 200 U/kg/week will cost the NHS £123 per week (or £6396 per annum).

The definition of a poor response to erythropoietin is arbitrary, but since most patients will respond to between 75 and 150 U/kg/week, any such patient showing a haemoglobin rise of less than 1 g/dl/month despite a dose of >200 U/kg/week may be classed as a 'poor responder'. Several factors have been shown to inhibit or prevent a response to erythropoietin [2,3], and these may be classified as 'major' and 'minor factors'. Major factors include iron deficiency (either 'absolute' or 'functional') [4,5], blood loss, which is often occult [6], and infection or inflammatory conditions, including malignancy [7,8]. Minor factors consist of hyperparathyroidism with marrow fibrosis [9,10], aluminium toxicity [11,12], vitamin B_{12} or folate deficiency [13], haemolysis [14], marrow dysfunction [15], red cell enzyme defects and haemoglobinopathies [16,17].

The aim of this article is firstly to discuss how each of these conditions might cause inhibition of a response to erythropoietin. Secondly, since there are few published guidelines on this subject, suggestions will be made on how best to investigate and manage each of these conditions. Finally, a clinical algorithm is offered as a guide to the possible management of the 'poor responder'.

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Causes of resistance to erythropoietin

Iron deficiency

This may be either 'absolute', which is defined as a reduction in total body iron stores; or 'functional', which implies adequate iron stores but a failure of supply of available iron to the marrow and/or its utilization in the process of erythropoiesis. It became evident in even the earliest clinical trials that large amounts of iron were consumed in the manufacture of new red cells under erythropoietin stimulation [18]. Many patients who had adequate iron stores at the start of treatment rapidly depleted these; other patients appeared to maintain adequate iron stores (as judged by the serum ferritin) but were unable to release iron from these stores rapidly enough to satisfy the requirements of the bone marrow. In both clinical situations, a blunted or absent response to erythropoietin is seen which can often be reversed by the administration of intravenous iron [4,5]. Iron deficiency may also be exacerbated by repeated phlebotomy for blood sampling, blood loss (see below), menorrhagia, and inadequate dietary iron intake due to the anorexia which many dialysis patients experience.

There is much controversy over how best to detect iron deficiency, and there is no ideal reliable marker. The serum ferritin [19,20] and transferrin saturation [21] (serum iron ÷ total iron binding capacity × 100%) are the most commonly employed, but both have drawbacks. A low serum ferritin (<30 μg/l) unequivocally indicates absolute iron deficiency. However, the threshold for iron deficiency in renal patients may be higher than in normal individuals, and cut-offs of 50 μg/l [22], 70 μg/l [23] and 80 μg/l [20] have been suggested. The problem with the use of serum ferritin is that a normal or even high level does not exclude functional iron deficiency [18]. The serum ferritin may also be spuriously raised in inflammatory conditions, infection, and liver disease [24,25]. Even in the absence of these complications, the serum ferritin is only an indicator of iron stores, and will give no guide as to

how much of this iron is available to the marrow for erythropoiesis. In theory, the transferrin saturation is in a better position to give this information since it reflects the circulating amount of iron in the plasma relative to the TIBC [21]. Previous studies have suggested that once the transferrin saturation falls below 16–20% then the iron supply for erythropoiesis will be inadequate [26]. The main problem with this measurement, however, is that it shows a marked diurnal variation which is entirely biological and not related to the assay used. Thus, even in normal subjects the transferrin saturation can vary from 15% to 70% depending on the time of sampling [27].

For these reasons, other indicators of functional iron deficiency in patients on EPO have been investigated, such as the red cell zinc protoporphyrin levels [28], red cell ferritin [28], percentage of hypochromic red cells in the circulation [29], serum transferrin receptor levels, stainable marrow iron, and ferrokinetic measurements. Of these, measurement of the % hypochromic red cells is perhaps the most useful [29] since this is an indirect measure of the adequacy of iron supply to the erythron and its incorporation into haemoglobin in the red cell. The test is also eminently practical as it can be performed rapidly on a routine full blood count sample; unfortunately, however, only some auto-analysers offer this facility. None of the other tests has proven ideal, due either to limited validation or availability of the techniques, or to the impractical and laborious nature of repeated measurements. For practical purposes, therefore, the serum ferritin and transferrin saturation remain the most widely used methods [30,31], and their use in this context will be discussed later.

Treatment of both absolute and functional iron deficiency is iron supplementation, which can be given either orally or intravenously, and again there is widespread debate among clinicians regarding the threshold for the use of intravenous iron [4,5,30,31]. In many patients oral iron supplementation is adequate to keep pace with the iron requirements [31], but in a significant proportion (which can vary from 10% to 60% depending on the reporting centre) of patients oral iron is insufficient and intravenous iron supplementation is required [1,4,5,32]. The reasons for this are unclear, but it would appear that iron requirements in patients receiving erythropoietin are often much greater than expected. Furthermore, although earlier studies suggested that oral iron is well-absorbed in dialysis patients with iron deficiency [33], this may not be the case in patients on erythropoietin therapy [34]. A randomized controlled study of iron supplementation in iron-replete (ferritin $>100\ \mu g/l$) patients receiving erythropoietin showed that the haemoglobin response was greater and EPO dosage requirements less in patients supplemented with intravenous iron compared with the groups receiving oral or no iron supplementation [35].

Blood loss

In addition to unavoidable losses caused by repeated blood sampling, patients with end-stage renal failure are at increased risk of occult gastrointestinal bleeding, partly due to a higher prevalence of gastritis and peptic ulceration [36], and partly due to an increased bleeding tendency due to both uraemic platelet dysfunction [37] and heparin administration during dialysis. Haemodialysis patients are also prone to variable blood losses in the dialyser [38]. A clue to intermittent blood loss is a sudden or dramatic drop in the haemoglobin concentration and/or heavy transfusion dependence, the only other cause of this being haemolysis. Another clue suggestive of blood loss or haemolysis is a significant reticulocytosis ($\geqslant 3\%$) in the absence of any rise in the haemoglobin concentration.

Investigation of occult blood loss has two goals: firstly, to confirm its presence, and secondly to ascertain the cause or site of gastrointestinal bleeding. Measurement of reticulocytes, faecal occult blood (FOB) testing, red cell life-span studies, and ^{59}Fe blood loss studies may all help to diagnose the presence of occult bleeding. The sensitivity of the non-quantitative FOB test is such that a positive result is often unhelpful; nevertheless three negative FOBs may be of some value in excluding significant gastrointestinal bleeding. A shortened red cell life-span using ^{51}Cr- or ^{59}Fe-labelled red cells indicates either bleeding or haemolysis, and ^{59}Fe blood loss studies (in which the patient's transferrin is labelled with ^{59}Fe which is then incorporated into the red cells) using a whole body counter can confirm unequivocally whether there are increased iron or blood losses. Using this technique, excessive blood losses have been demonstrated in dialysis patients both off [39] and on [40] erythropoietin treatment, although it remains largely a research tool rather than a routine investigation.

Having suspected occult bleeding, and after excluding other obvious sources of blood loss, a decision must be made as to how far to take further investigation of the gastrointestinal tract. Extensive investigation including upper gastrointestinal endoscopy, sigmoidoscopy, proctoscopy, barium enema ± colonoscopy, and small bowel enema is often unrewarding, and probably many such cases have slow generalised oozing from small bowel mucosa as part of their uraemic bleeding tendency [37]. Even if (as is often the case) one finds mild/moderate gastritis on endoscopy, there must still be some doubt as to whether this is the major source of blood loss. Underinvestigation, however, runs the risk of failing to detect an early gastrointestinal malignancy. Empirical treatment with H_2 receptor blockers or omeprazole is often worthy of consideration.

Inflammation/Infection/Malignancy

Patients with acute [8] or chronic [7,41] infection, inflammatory disease [42], or malignancy [8] frequently show remarkable resistance to the effects of erythropoietin, and often this cannot be overcome even with very large doses of the drug. The mechanism of this effect is unclear, but is almost certainly the same as that which causes the anaemia of chronic disease

[43]. Two, possibly overlapping, mechanisms may be involved. Firstly, there is evidence of a disturbance of iron metabolism, and in particular there appears to be a failure of iron release from its storage sites in the reticulo-endothelial system (RES blockade). There is evidence of reduced iron absorption from the gut, increased plasma clearance of iron, and increased ferritin synthesis in the RES; thus iron accumulates in its storage sites, and is unable to be mobilized to supply the bone marrow for erythropoiesis [44].

Secondly, there is evidence of suppression of erythropoiesis by humoral factors. In 1984, it was found that serum from patients with rheumatoid arthritis suppressed erythroid colony growth in tissue culture [45]. Since then, it has become apparent that a number of cytokines and growth factors can modify erythropoiesis *in vitro* [46]. Thus, IL-1α, TNFα, and IFNγ have been shown to be inhibitory, while IL-3 and IGF-I potentiate erythropoiesis. Since circulating levels of a number of cytokines are known to be elevated in inflammatory disease states [46], it is quite possible that one or more of these factors may directly or indirectly modify the action of erythropoietin at the cellular level.

The cause of the inflammatory condition producing resistance to erythropoietin may be obvious (such as bronchopneumonia [8], septicaemia, CAPD peritonitis, active rheumatoid arthritis) or occult (such as unsuspected vasculitis [42], osteomyelitis [41], malignancy [8], or ongoing low-grade rejection in a renal transplant [47]). Investigation should be directed at confirming and quantitating the severity of the inflammatory condition using markers such as C-reactive protein [48], plasma viscosity, or serum IL-6 levels [49] (the ESR is often spuriously elevated in renal failure and is therefore unreliable [50]), and detecting the underlying cause. If the latter is not obvious then the list of investigations included in Table 1 may prove useful. Overcoming the resistance to EPO in most inflammatory disease states will require reversal of its cause, and although an initial doubling of the dose of EPO is reasonable, further and repeated dose increments are unlikely to be successful. Graft nephrectomy should be considered in any patient with a failing renal transplant [47,49], 'blind' treatment with broad-spectrum antibiotics may be an option in some patients (e.g. those with possible infected liver or renal cysts [49]), and a trial of steroids might be considered in any patient with undiagnosed inflammatory disease where infection has been carefully excluded.

Hyperparathyroidism/marrow fibrosis

Several studies have investigated the effect of hyperparathyroidism on EPO responsiveness [9,10,51], and there is some controversy over whether excessive parathyroid activity *per se* causes resistance to EPO. An improvement in anaemia has been demonstrated in some dialysis patients following sub-total parathyroidectomy [52]. Furthermore, early *in vitro* studies showed that a crude extract of parathyroid tissue could inhibit the growth of erythroid progenitor cells in culture [53], although the majority of clinical studies have since failed to confirm this interaction *in vivo* [9,10,51]. However, patients who have severe hyperparathyroidism with osteitis fibrosa do show considerable resistance to erythropoietin due to replacement of the cellular components of the marrow by fibrous tissue [10]. Investigation of this condition includes measurement of serum PTH, calcium, phosphate, alkaline phosphatase levels, skeletal radiology and, on occasion, bone marrow biopsy. Treatment consists of intravenous calcitriol or parathyroidectomy, but the marrow fibrosis, if present, is irreversible.

Aluminium toxicity

Excessive plasma and bone aluminium levels in dialysis patients are becoming less common with the widespread use of deionizers and the decreasing trend in the use of aluminium-containing phosphate binders. Aluminium toxicity on its own causes a microcytic anaemia [54], and it has been shown in several studies to cause resistance to erythropoietin [9,11,12,55]. The mechanism of this effect is only partly understood, but factors believed to be responsible include interference with iron transport and/or utilization, inhibition of haem synthesis, and increased haemolysis due to an increase in red cell fragility [56]. Measurement of the serum aluminium level alone is unreliable, and if this condition is suspected then a desferrioxamine challenge test or bone biopsy may be required. Treatment of aluminium toxicity is by withdrawal of aluminium-containing phosphate binders, and by repeated intermittent desferrioxamine chelation therapy.

Vitamin B_{12}/folate deficiency

Deficiencies of either vitamin B_{12} or folic acid will result in ineffective erythropoiesis, usually with a megaloblastic marrow and macrocytic red cells in the peripheral blood. In practice, however, this has very rarely been found to be a problem in patients receiving erythropoietin [13], in contrast to iron deficiency. Its ease of detection (by measuring serum B_{12} and folate, or red cell folate, levels) and treatment (by oral folic acid or parenteral B_{12} supplementation) means that this condition must be excluded in patients responding poorly to erythropoietin, particularly if the mean red cell volume (MCV) is raised.

Haemolysis

As with occult blood loss, features suggestive of haemolysis include rapid falls in the haemoglobin after transfusion, heavy transfusion dependence, and an enhanced reticulocyte response in the absence of any haemoglobin rise. The blood film may show fragmented red cells, and other tests which might be useful include a Coombs test, serum haptoglobin measurement, serum lactate dehydrogenase, G6PD level, acid lysis test, and red cell fragility studies. Current and recent medications should be scrutinised and any drug known to

Table 1. Factors causing a poor response to EPO

	Investigation	Management
Major factors		
Iron deficiency	serum ferritin transferrin saturation % hypochromic red cells red cell zinc protoporphyrin	Iron supplementation – oral (ferrous sulphate) – IV (iron dextran)
Blood loss (?occult)	FOBs endoscopy barium enema/colonoscopy	H_2 blockers/omeprazole
Inflammation/infection/malignancy	CRP, PV, IL-6 levels blood cultures MSU ANF, Rh factor, ANCA viral titres Mantoux test CXR abdo US/CT scan bone scan ? echocardiogram ? bone marrow (for myeloma)	Treat underlying cause (if appropriate) ? blind trial of ciprofloxacin ? blind trial of steroids ? graft nephrectomy
Minor factors		
Hyperparathyroidism/marrow fibrosis	serum PTH bone marrow biopsy	Vitamin D/calcium supplementation ? Parathyroidectomy
Aluminium toxicity	serum aluminium DFO test ? bone biopsy	Desferrioxamine chelation therapy
B_{12}/folate deficiency	B_{12}/folate levels	B_{12} injections IM Folic acid orally
Haemolysis	retics, blood film LDH, bilirubin Coombs test, G6PD screen haptoglobin	? Steroids
Marrow dysfunction	bone marrow biopsy	–
Red cell enzyme defects/ haemoglobinopathies	Hb electrophoresis sickle cell test	–

Abbreviations: FOB_s, faecal occult blood tests; CRP, C-reactive protein; PV, plasma viscosity; IL-6, interleukin-6; MSU, midstream specimen of urine; ANF, anti-nuclear factor; Rh factor, rheumatoid factor; ANCA, anti-neutrophil cytoplasmic antibody; CXR, chest X-ray; abdo US, abdominal ultrasound; abdo CT, abdominal computerized tomography scan; PTH, parathyroid hormone; DFO, desferrioxamine; LDH, lactate dehydrogenase; G6PD, glucose-6-phosphate dehydrogenase; IV, intravenously; IM, intramuscularly.

cause oxidant stress to red cells should be stopped. The possibility of immune-mediated haemolysis induced by residual formaldehyde in reused dialyzers should be considered [14]. A shortened red cell lifespan coupled with normal ^{59}Fe loss studies would confirm the diagnosis, but both these tests are laborious, time-consuming, and expensive. The treatment consists of removing the cause of haemolysis if possible, and/or steroids if immune-mediated (Coombs positive).

Marrow dysfunction

There are a number of conditions affecting the marrow which may result in ineffective erythropoiesis and which are refractory to erythropoietin stimulation (myelodysplastic syndrome, aplastic anaemia, marrow infiltration by tumour, advanced multiple myeloma, etc.) [15]. The diagnosis is confirmed by bone marrow aspirate and/or trephine biopsy. Initial concerns arose over whether EPO therapy might increase the risk of malignancy in myelodysplastic syndrome (a pre-malignant condition) or worsen multiple myeloma by stimulating the malignant cell clone, but apart from one worrying report [57] there has been little to substantiate such fears. Provided the disease is neither too advanced nor too active, EPO appears to be effective in patients with myeloma and renal failure [58,59].

Red cell enzyme defects/haemoglobinopathies

The response to EPO in patients with renal anaemia complicated by sickle cell disease [17,60], other haemoglobinopathies [16,61], or a red cell enzyme defect [62] has been variable. Patients with a mild form of thalassaemia (β minor or α trait) appear to respond quite well, but often require much larger doses of EPO [16,61]. Those with sickle cell disease have unfortu-

nately fared less well, and although it has been possible to demonstrate evidence of increased erythropoiesis (a rise in reticulocyte count and HbS levels) [17], an improvement in the degree of anaemia has been lacking [17,60]. It may however be possible to reduce the frequency of blood transfusion in sickle cell patients receiving EPO, and so limit excessive iron overload. Red cell enzyme defects are much less common, but pyruvate kinase deficiency has been reported to cause resistance to EPO [62]. Screening for a haemoglobinopathy by a sickle cell test or haemoglobin electrophoresis should be mandatory in any patient whose ethnic background suggests this as a possibility. Unfortunately, however, EPO is unable to correct the inherent genetic defects involved in haemoglobin synthesis.

Investigation and management

What should be done when faced with a patient who is responding poorly to EPO? The following practical guidelines and algorithms (Figures 1 and 2) are offered as a suggested approach to this important clinical problem. They are based to a large extent on the author's personal experience but with due consideration of relevant published work.

The first two important questions to consider are (i) is the patient complying with the treatment (if self-injecting)? and (ii) is it possible that the patient has iron insufficiency?

For reasons already discussed, it may be difficult to exclude conclusively the presence of functional iron deficiency, and if there is doubt, then a trial of intravenous iron supplementation (with careful monitoring of the haemoglobin and reticulocyte count) is worthwhile. As indicated above, it is impossible to be precise regarding exact cut-offs for serum ferritin and transferrin saturation but a suggested approach is shown in Figure 1.

If the patient is receiving, and complying with, an EPO dose of $\geqslant$200 U/kg/wk, and iron insufficiency has as far as possible been excluded or corrected, then a number of first-line investigations are indicated to look for another cause of EPO resistance (Figure 2).

Measurement of C-reactive protein (CRP) is probably the best screen for underlying infection or inflammatory disease; an alternative is plasma viscosity, and both are superior to measurement of the ESR which is of limited use in renal failure [50]. If the CRP is $<$10 mg/l, significant inflammatory disease causing suppression of erythropoiesis is extremely unlikely. The CRP is also useful in monitoring the progress of, and/or recovery from infective or inflammatory conditions, although it often seems to lag behind clinical recovery by several weeks. If the CRP is raised and there is no overt infection or inflammatory disease, then it becomes necessary to search for occult disease by means of a number of second-line investigations (Figure 2).

A significant reticulocytosis ($>$3–4%) in the absence of a haemoglobin response suggests either blood loss or haemolysis, all other causes of EPO resistance yielding a low reticulocyte count. A blood film (for fragmented red cells) and a Coombs test may confirm the presence of haemolysis; a raised serum bilirubin or lactate dehydrogenase level is suggestive. The value of testing for faecal occult blood in this context is debatable; three negative results make significant gastrointestinal blood loss unlikely. Three positive results along with a significant reticulocytosis, absent haemoglobin response, and negative haemolysis screen probably merit further investigation of the gastrointestinal tract and/or a trial of H_2 receptor blockers or omeprazole (Figure 2).

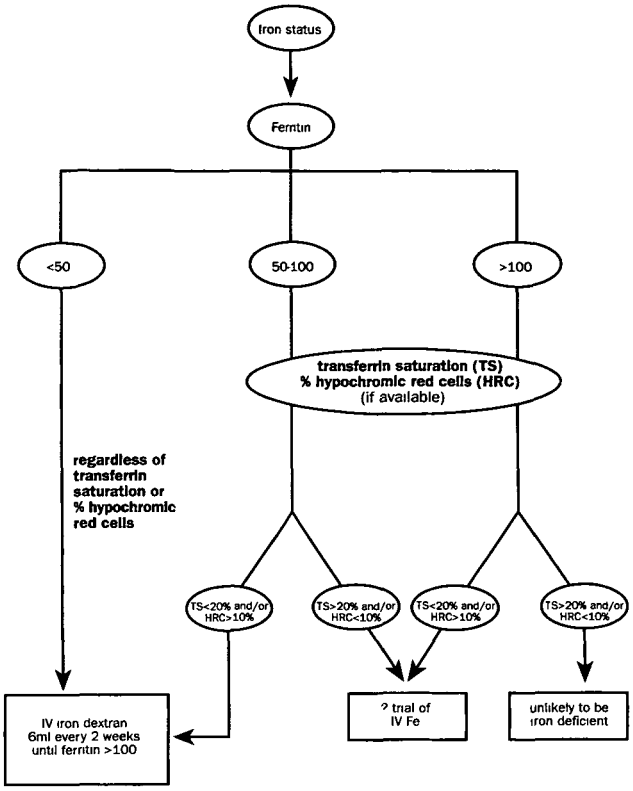

Fig. 1. Investigation of the *Poor Responder* to EPO (1).

Measurement of the serum PTH level will give a reasonable indicator of the severity of hyperparathyroidism; a grossly elevated level may merit consideration of a bone marrow trephine biopsy to assess the degree of marrow fibrosis since if this is severe then EPO is unlikely to be effective [10] and should probably be stopped. A bone marrow biopsy should also be considered in all patients in whom a cause of poor response cannot be identified [15], in order to assess the adequacy of erythroid precursor tissue and to screen for conditions such as myelodysplasia.

In the absence of gastrointestinal tract disorders such as Crohn's or coeliac disease, it is extremely rare to find low B_{12} or folate levels as a cause of poor response to EPO, but since deficiencies of these vitamins are easily detected and treated they should be

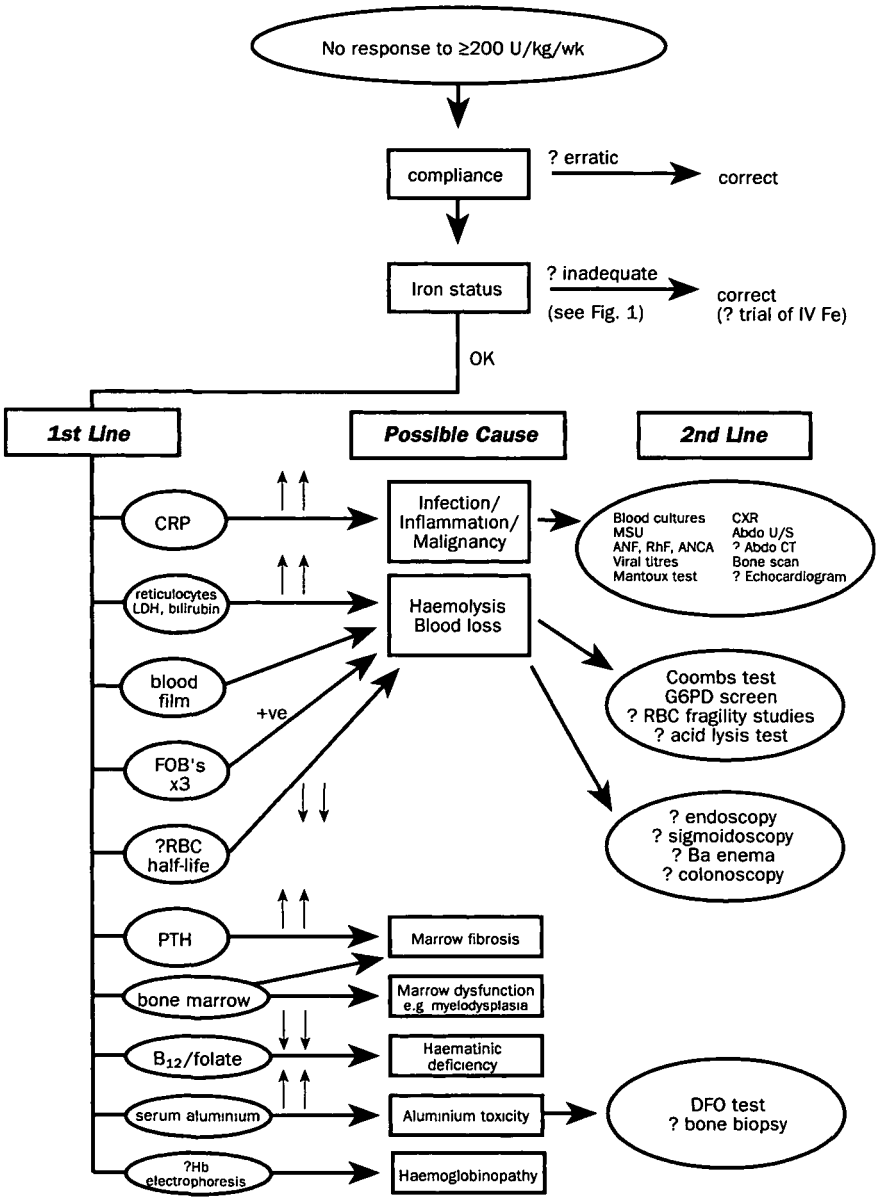

Fig. 2. Investigation of the *Poor Responder* to EPO (2).

excluded. Likewise, in patients who have been on dialysis for many years and/or who have used aluminium-containing phosphate binders, the serum aluminium level before and after desferrioxamine should be measured. If there is a significant increment following desferrioxamine, then long-term chelation therapy with this drug should be used as an adjunct to EPO.

The presence of sickle cell disease or another haemoglobinopathy is usually apparent before starting EPO, but in patients in whom haemoglobin electrophoresis has not previously been performed this should be done, particularly if their ethnic background merits it.

In a patient in whom erythropoiesis is suppressed by infection or inflammatory disease, one of the most difficult decisions is what to do about the dose of EPO. Some centres in the UK will stop the EPO altogether until such an infective episode is treated, while others will increase the dose to very high levels with no effect, but incurring considerable cost. The problem with complete withdrawal of therapy is that the haemoglobin often falls to levels even lower than at the start of treatment, and there is often difficulty in subsequently re-establishing a response to EPO. The mechanism of this effect is unexplained, but may involve suppression of endogenous erythropoietin production by EPO therapy, analogous to adrenal suppression by exogenous steroids. A reasonable compromise would seem to be to continue the same dose of EPO throughout the infective episode (accepting that a blood transfusion may also be required) and wait for the haemoglobin response to be restored, which may be several weeks after the clinical recovery.

Conclusions

Resistance to erythropoietin therapy is an important and not uncommon finding in patients receiving this treatment. It may be transient and reversible (e.g. when associated with an acute infective or bleeding episode) or permanent and irreversible (e.g. when associated with marrow fibrosis or some haemoglobinopathies). Identification of the cause is not always easy, and multiple factors may be contributing. Nevertheless, every attempt should be made to investigate thoroughly any patient with erythropoietin resistance, particularly since the treatment is expensive and some causes are easily corrected. Hopefully, as our understanding of erythropoiesis advances, and the contribution of other cytokines and growth factors in this process is elucidated, alternative therapeutic options might become available which could increase erythropoietin responsiveness.

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