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Anaemia in Cancer
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Erythropoietic Agents and Iron

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Introduction

Patients with solid tumours or haematological malignancies often develop anaemia at diagnosis or in the course of the disease [1–7]. Many studies have shown that treatment with erythropoietic agents, such as recombinant human erythropoietin (rHuEpo) or darbepoetin-alpha, can ameliorate the anaemia associated with cancer and chemotherapy, reduce the need for transfusions and improve quality of life [4,8]. This treatment is effective in about two-thirds of cancer patients. A number of disease- or chemotherapy-related factors determine the probability of response [9–11]. Several specific mechanisms of anaemia, such as haemolysis, splenomegaly, bleeding, haemodilution or ineffective erythropoiesis can seriously interfere with response, and unforeseen events, such as surgery or infections, may induce temporary loss of response. However, another important response-limiting factor is probably functional iron deficiency, i.e., an imbalance between iron needs in the erythropoietic marrow and iron supply, which depends on the level of iron stores and its rate of mobilisation [12]. Functional iron deficiency has been recognised for many years by nephrologists who now apply a policy of systematic intravenous (i.v.) iron supplementation [13]. This policy has been shown to result in improved efficacy of erythropoietic agents [14], as well as in substantial cost savings because of consequential erythropoietin dose reductions [15,16]. The need for adequate iron supply has been emphasised in other clinical situations in which rHuEpo therapy is used [17]. Surprisingly, haematologists and oncologists have so far mostly ignored the question of iron requirements during rHuEpo therapy in

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cancer patients [13]. The medical literature scarcely addresses the topic, so that very few data on iron metabolism and iron therapy are available in these patients. In this paper, we will first review iron metabolism, a field that has changed dramatically over the past 10 years following the discovery of many new proteins and concepts [18,19], and subsequently summarise its abnormalities in cancer patients. We will then review the biological tools available for evaluating the iron status of patients and define how these parameters can be used to detect absolute (exhaustion of iron stores) and functional (erythroid marrow iron deficiency despite adequate iron stores) iron deficiency. Finally, we will situate the possible impact of functional iron deficiency among other factors potentially affecting response to erythropoietic agents. Although, while awaiting the results of ongoing clinical trials, no prospective comparative data are available to provide evidence-based recommendations, the clinical experience in renal failure patients as well as other situations can provide some guidelines for iron supplementation in cancer patients undergoing treatment with erythropoietic agents. Therefore, preferably intravenous iron supplements should be given when serum ferritin is below 40–100 µg/l, reflecting the absence of iron stores, or when the transferrin saturation is below 20%, or the percentage of hypochromic red cells is above 10%, indicating functional iron deficiency even in the presence of adequate storage iron (normal or increased ferritin).

Iron metabolism

Body iron exchange

The human body contains 30–40 mg/kg of iron, adding up to approximately 4 g in adults [19–21]. Iron is mostly contained in haemoglobin (2.5 g), ferritin (1 g) and other haeme and non-haeme proteins (0.5 g), while plasma iron amounts to only 3 mg. Iron exchanges are very limited. There is no active iron excretion mechanism and iron losses are limited to about 1 mg/d through gastrointestinal and skin desquamation. A normal western diet provides 10–15 mg of iron daily but only a small fraction, 1 mg in a male adult, is absorbed. Iron requirements depend on age (1.5 mg/d during puberty), sex (1.5 mg in women because of menstrual losses) and pregnancy (3.5 mg/d and even 5 mg/d in the third trimester).

Iron absorption by the gastrointestinal tract depends on several factors, including iron bioavailability [22,23]. Haeme iron is better absorbed than non-haeme iron whose absorption is reduced by low gastric acidity and a number of other nutrients that interfere with absorption [22]. Iron import at the apical surface of enterocytes is carried out by a protein called DMT1 (dimetal transporter 1) or Nramp2 (natural resistance-associated macrophage protein 2), but to be actively transported iron must first be reduced by a ferric reductase associated with DMT1 (Figure 1) [20,24]. Within enterocytes, iron can either be

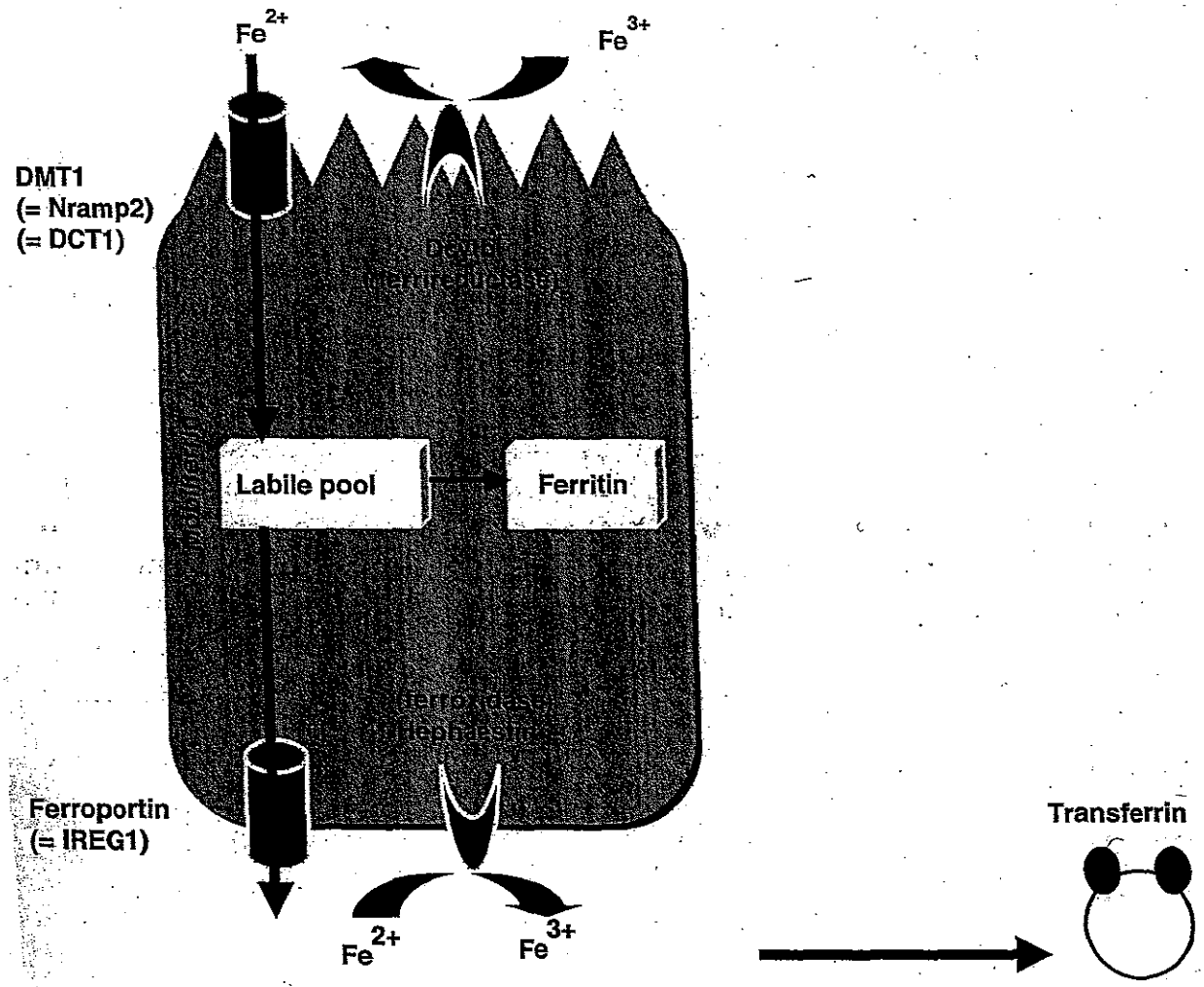

Fig. 1. Scheme of iron absorption. Iron import at the apical surface of enterocytes is carried out by a protein called DMT1 (dimetal transporter 1) or Nramp2 (natural resistance-associated macrophage protein 2), but to be actively transported iron must first be reduced by a ferric reductase, duodenal cytochrome b (Dcytb), associated with DMT1. Within enterocytes, iron can either be stored in the form of ferritin or enter the so-called labile iron pool. Then iron can be actively exported at the basolateral surface of enterocytes by another protein called ferroportin. Ferroportin is also associated with another protein, hephaestin, that oxidises ferrous iron back to ferric iron, allowing its tight binding to circulating transferrin.

stored in the form of ferritin (and later excreted through cell exfoliation) or enter the so-called labile iron pool whose biochemical nature remains poorly defined [22]. Then iron can be actively exported at the basolateral surface of enterocytes by another protein called ferroportin. Ferroportin is also associated with another protein named hephaestin that oxidises ferrous iron back to ferric iron, allowing its tight binding to circulating transferrin [20].

Iron absorption is largely regulated by the level of iron stores and marrow erythropoiesis [23]. Increased erythropoietic activity, such as encountered in chronic dyserythropoietic anaemia or thalassaemia, results in progressive iron loading independently of transfusions, through mechanisms that remain

obscure. The regulation of iron absorption by tissue iron stores could be mediated by plasma transferrin saturation. Indeed diferric transferrin is taken up by receptors at the basolateral pole of enterocytes, thereby increasing the labile iron pool and thus repressing expression of DMT1. Another protein, called HFE, has been cloned [25]. While a typical mutation of this protein is responsible for dysregulated iron absorption associated with genetic haemochromatosis, its exact function remains unclear [25]. However, by interacting with both transferrin receptor and β2-microglobulin at the basolateral surface of enterocytes, the HFE protein somehow plays an important role in regulating iron absorption. The recent discovery of hepcidin, a plasma protein secreted by hepatocytes in response to iron, could provide the missing link between marrow erythropoietic activity and iron stores on the one hand, and the level of iron absorption on the other [26]. Indeed hepcidin levels are increased in cases of inflammation or iron overload (two situations associated with decreased iron absorption) and elevated in cases of anaemia, hypoxia or iron deficiency (all associated with increased absorption). However, the putative molecular events triggered by hepcidin in enterocytes remain unknown [27].

Iron transport

Iron is transported in the plasma by transferrin, which possesses two equivalent binding sites, providing for the three forms of diferric transferrin, monoferric transferrin and apotransferrin [21]. When transferrin is nearly saturated, a small amount of highly toxic non-transferrin-bound iron (NTBI) may be encountered [21]. Iron enters cells through interaction of plasma transferrin with the transferrin receptor 1 (TfR) [28,29]. The TfR is a dimeric protein with two identical monomers of 90,000 daltons linked by two disulphide bonds. After binding to the TfR, the transferrin–TfR complex is internalised in endosomes, iron is released after acidification of the endosomes and transported to the cytoplasm by DMT1, and the TfR re-integrates the cell wall while apotransferrin is released back to the plasma [20,28,29]. In the cytoplasm, iron enters the labile pool before being transported to mitochondria that incorporate it into haeme and other proteins [20,30].

Virtually all cells, except mature red cells, possess TfR, but the highest density is observed on erythroid precursors in the bone marrow, including erythroblasts (800,000 TfR per cell) and reticulocytes (100,000 per cell) [30]. In a normal individual, approximately 80% of body TfR are located in the bone marrow, but this proportion increases when erythropoiesis is stimulated (Figure 2) [31].

The major pathways of iron transport have been identified [32]. The main circuit involves transferrin iron uptake by the erythroid marrow and its incorporation into red cell haemoglobin, where its stays until phagocytosis of senescent red cells by macrophages. Macrophages digest haemoglobin and iron enters the labile iron pool. Thereafter, two-thirds are rapidly ($t_{1/2}$ 30 minutes) released

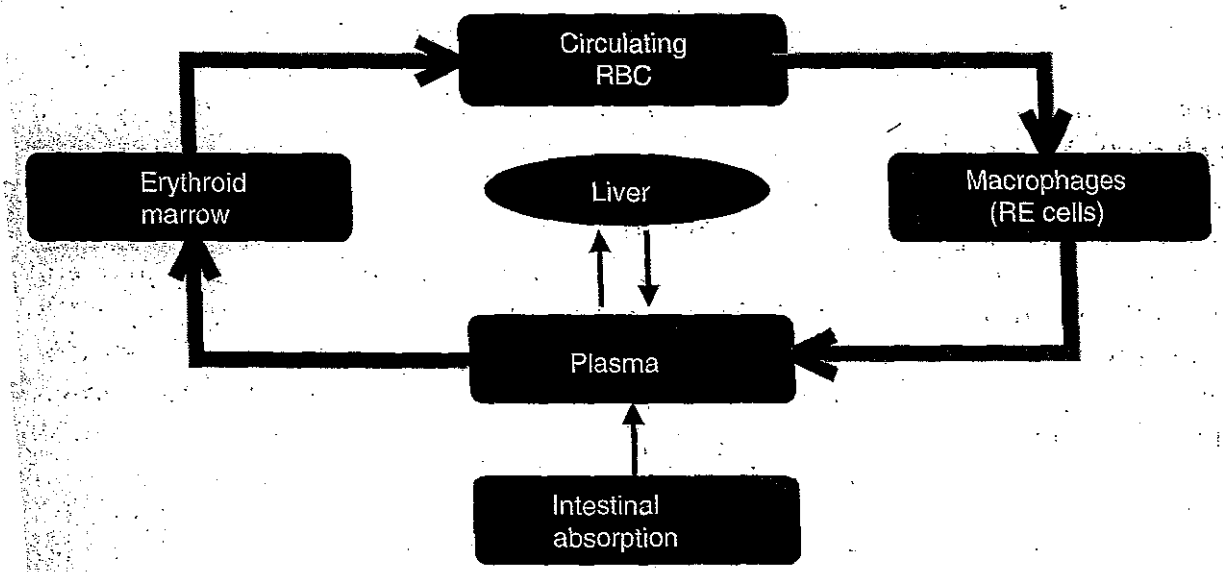

Fig. 2. Main pathways of iron metabolism. After absorption by the gastrointestinal tract, iron is taken up by transferrin that transports it for uptake by the erythroid marrow and incorporation into red cell haemoglobin, where its stays until phagocytosis of senescent red cells by macrophages. Macrophages digest haemoglobin and iron is released back to plasma transferrin. A minor fraction of transferrin iron is available for uptake by transferrin receptors in other tissues, in particular hepatocytes.

back to plasma transferrin and one-third is incorporated into intracellular ferritin from which it can be slowly ($t_{1/2}$ 7 days) released to transferrin [33]. A second circuit involves iron uptake by different tissues, including hepatocytes, and a third one involves the circulation of transferrin iron in extravascular spaces [32]. On the contrary to other tissues, iron exchanges with hepatocytes are bidirectional.

Intracellular iron

The regulation of intracellular iron is exerted by a reciprocal control of the synthesis of ferritin and TfR [34]. Messenger RNAs of these two proteins contain in their untranslated segments loop structures called IREs (iron regulatory elements) to which cytoplasmic IRPs (iron regulatory proteins) can bind [29]. IRP1 can either be a factor regulating translation or an enzyme (aconitase), depending on the absence or presence of an iron–sulphur centre. When iron is lacking, IRP1 can bind to IREs of ferritin and TfR mRNAs: ferritin mRNA cannot be translated while TfR mRNA is stabilised, thereby resulting in increased iron acquisition through TfR and decreased iron storage in ferritin. When iron is abundant, IRP1 retains its iron–sulphur centre that prevents such binding: ferritin mRNA can be translated and TfR mRNA is degraded, thereby resulting in diminished iron uptake and increased ferritin storage. Additional factors contribute to the regulation of TfR. There is transcriptional regulation facilitated by

mitogens, erythropoietin or erythroid differentiation, as well as post-transcriptional regulation by NO [35,36], a molecule also involved in the post-transcriptional regulation of ferritin expression [37]. IRP1 can also be activated by some cytokines [35,36].

Two types of cells are particularly involved in iron storage, namely hepatocytes and macrophages/monocytes (reticuloendothelial system). Iron stores accumulate in the form of ferritin and also haemosiderin, an aggregated and partially denatured form of ferritin [34]. Ferritin is composed of 24 subunits of apoferritin arranged as an empty shell containing an iron core with 0–4500 atoms of iron. Serum ferritin is produced in proportion to the amount of intracellular ferritin and thus represents a quantitative marker of iron stores even if it does not contain iron itself.

Iron metabolism in cancer

Anaemia in cancer patients

The anaemia observed in cancer patients has multiple mechanisms [2,3,38]. Haemodilution may artificially dilute the red cell mass [39]. Bleeding, autoimmune or microangiopathic haemolysis, hypersplenism and haemophagocytosis may all reduce the red cell life span. Nutritional deficiencies, including iron, folate, vitamin B12 and global malnutrition may impair red cell production. The bone marrow may be involved by metastases, necrosis, myelodysplasia and autoimmune red cell aplasia, or be further altered by chemotherapy and radiotherapy. These various causes have been reviewed in detail elsewhere [40].

Neoplastic disorders, however, are often complicated by the "anaemia of chronic disease" (ACD), defined as the anaemia associated with infection, inflammation, cancer or trauma, that has the characteristic picture of hypoferraemia, hyperferritinaemia, decreased transferrin concentration and increased iron stores. The pathogenesis of ACD remains unclear but may involve the combination of a shortened erythrocyte survival in circulation [41] with failure of the bone marrow to increase red cell production in compensation [1,42–44]. Inappropriate red cell production is itself related to a combination of factors, including impaired availability of storage iron, inadequate erythropoietin response to anaemia and overproduction of cytokines which are capable of inhibiting erythropoiesis [1–3,42,43,45]. Increased production of several cytokines, including IL-1 [46], TNF [47], IFN-γ [48], TGF-β [49] and IL-6 [50] has been demonstrated in a variety of cancers. In particular, TNF [51] and IL-6 [52] plasma levels have been found to be elevated in some cancer patients. These cytokines [1,35,42,45] are involved in the retention of iron in the reticuloendothelial system, gastrointestinal tract and hepatocytes [35,36,53–55], may interfere with erythropoietin production by the kidney and may exert direct inhibitory effects on erythroid precursors.

Iron metabolism in cancer

As reviewed elsewhere, iron plays an important role in the growth of malignant cells which often express high numbers of transferrin receptors and are capable of synthesising excess amounts of ferritin [53,56–58]. There is no indication that iron supplementation may interfere with the effect of chemotherapy, but it has been suggested that iron withholding from tumour cells may represent a defence system against tumour growth [59,60]. Iron metabolism is considerably altered in the anaemia of chronic disease (ACD) [35,36,54,55] and particularly in cancer patients [53,57]. The main picture is one of iron-deficient erythropoiesis in the face of normal or increased iron stores. Serum iron and transferrin are decreased, while serum ferritin is often elevated [53,55]. As iron supply to erythroblasts is inadequate, red cell protoporphyrin is often increased [61]. However, serum levels of soluble transferrin receptor are not increased in patients with ACD and this may help distinguish them from patients with iron deficiency [31,62].

The mechanisms responsible for the hypoferraemia and the subsequent limitation of iron supply are multiple. In cancer patients, intestinal iron absorption is impaired [63], storage iron release by hepatocytes is diminished [64] and cells of the reticuloendothelial system also retain iron. Indeed, although some studies in tumour-bearing rats did not show it [65], the latter was clearly demonstrated by ferrokinetic studies using a tracer dose of heat-damaged ^{59}Fe-labelled red cells in man [33] as well as in animals with implanted carcinoma [66]. Although iron supply is clearly limited, there is no alteration in the way the erythroid marrow acquires plasma iron [33,67].

Several cytokines could reproduce at least part of the picture of the anaemia of chronic disorders. Interleukin-1 (IL-1) causes hypoferraemia with increased storage iron [68,69] at least in part through inhibition of reticuloendothelial iron release [70]. Other studies suggest that TNF also causes hypoferraemia through inhibition of macrophage iron release [71,72]. IL-6 also induces hypoferraemia [73] by increasing transferrin iron uptake and ferritin expression by hepatocytes but not Kupffer cells [74]. The mechanism accounting for the retention of iron into reticuloendothelial stores appears to exist in repressed TfR expression as well as stimulated ferritin synthesis controlled by transcriptional as well as post-transcriptional mechanisms [75,76]. Several cytokines may be involved [53,54,77], particularly IL-1 [78], TNF-α [79–81] and IFN-γ [80,82–84]. In addition, NO may be a mediator for the actions of several inflammatory cytokines [85], in particular IFN-γ [82,83].

Clinical tools for the evaluation of iron metabolism

Transferrin saturation

Serum iron saturates serum transferrin to a certain degree and transferrin saturation is a reflection of the equilibrium between iron supply and iron usage

(Figure 3) [86]. Iron needs are mostly represented by erythroid marrow activity while iron supply is almost exclusively determined by macrophage iron release. Serum iron and transferrin saturation will therefore decrease when the demand for iron is increased (increased erythropoiesis, for instance during erythropoietin therapy) or when iron supply is impaired (iron deficiency or inflammatory iron blockade). Serum iron and transferrin saturation are also subject to circadian fluctuations, the highest values being recorded around 11 am. This does not, however, significantly interfere with the detection of functional iron deficiency by low transferrin saturation.

Serum ferritin

Serum ferritin levels are directly proportional to storage iron levels in macrophages and hepatocytes [87]. Serum ferritin is a highly specific marker, any decreased value demonstrating exhaustion of iron stores. It is, however, not very sensitive, because numerous conditions are associated with falsely elevated serum ferritin levels. These include hepatic cytolysis, inflammation, renal failure, hyperthyroidism, poorly-controlled diabetes mellitus, some tumours and the rare hyperferritin-cataract syndrome. Indeed cut-off values for iron deficiency can be as high as 40–120 µg/l instead of the classical 12 µg/l in situations such as renal failure or inflammation.

Soluble transferrin receptor

A soluble form of the TfR (sTfR), a truncated monomer of the cellular TfR, circulates in the plasma and its concentration is directly proportional to the total body mass of cellular TfR [31]. It is therefore largely influenced by the level of erythropoietic activity (through changes in the number of erythroblasts) and to a lesser extend by iron stores (through regulation of the number of TfR per cell) [31]. However, as the impact of erythropoiesis quantitatively predominates, sTfR cannot be used as a marker of iron deficiency in erythropoietic disorders or during treatment with rHuEpo [31]. Otherwise, it is an excellent indicator of iron-deficient erythropoiesis [88,89]. It can be very useful for the differential diagnosis of iron deficiency (increased sTfR and low ferritin) vs inflammation (normal sTfr and ferritin) or for detecting iron deficiency in a patient with concomitant inflammation (increased sTfR and normal ferritin) [90].

Other parameters

Additional parameters may help in the diagnosis of iron-deficient erythropoiesis. Red cell protoporphyrin increases when erythroblasts lack iron for haeme synthesis [61]. The percentage of hypochromic red cells, individually

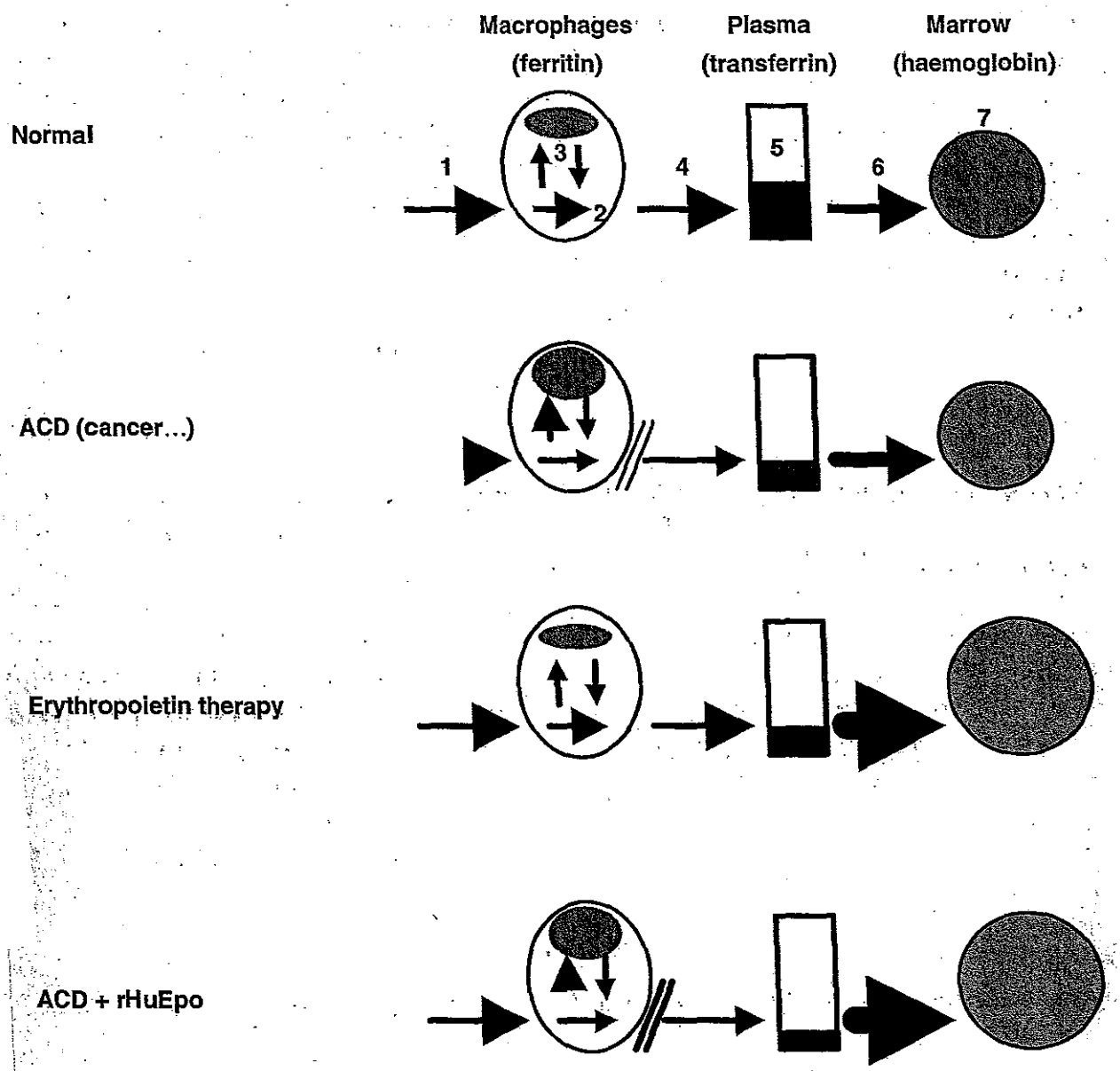

Fig. 3. Iron metabolism in conditions illustrating functional iron deficiency. *Normal*: when senescent red cells are phagocytosed (1) by macrophages, iron is recycled into a transit pool (2); part is stored as ferritin (hatched area) (3) and the rest is released (4) to plasma transferrin (5); iron is then taken up (6) by the erythroid marrow (7) to produce normal red cells. Iron supply (4) by storage cells matches iron demand (6) by the erythroid marrow and transferrin remains adequately (20–40%) saturated (black shading) by iron (5). *Anaemia of chronic disorder (ACD)*: iron release by macrophages is blocked and more iron is stored as ferritin within these cells. Iron supply can no longer match iron demand by the erythroid marrow: transferrin saturation decreases (<20%), the erythroid marrow becomes functionally iron deficient and new red cells are hypochromic. *Treatment with rHuEpo*: the erythroid marrow expands upon intense stimulation by erythropoietin. Its increased demand for iron cannot be matched by storage iron release; transferrin saturation decreases (<20%), the erythroid marrow becomes functionally iron deficient and new red cells are hypochromic. *ACD treated with rHuEpo*: impaired iron supply and increased iron demand combine to decrease transferrin saturation and cause functional iron deficiency.

characterised by cell haemoglobin concentration below 28 g/dl, increases over the upper limit of 5% when erythroblasts are deprived of adequate iron supply [91]. However, as reticulocytes are 20% bigger than mature red cells for the same amount of haemoglobin, they are also hypochromic [92]. Hence, high reticulocytosis should be taken into account in the interpretation of the percentage of hypochromic red cells [93]. The haemoglobin content of reticulocytes (CHr) will also decrease when the marrow does not receive enough iron to match its requirements [91,94]. As reticulocytes have a much shorter life span than red cells, the CHr will change much more rapidly than the percentage of hypochromic red cells or red cell protoporphyrin following the recent onset of iron-deficient erythropoiesis [95,96].

Absolute vs functional iron deficiency

Absolute iron deficiency

Absolute iron deficiency is defined by the exhaustion of iron stores in macrophages and hepatocytes [97]. One can distinguish three successive stages of iron deficiency (Table 1). Stage 1 corresponds to the progressive exhaustion of iron stores but without significant effect on marrow erythroid function. This situation is characterised by serum ferritin values decreased below the normal range. Serum ferritin is an excellent marker of storage iron and a decreased value is 100% specific for iron deficiency. Stage 2 corresponds to early signs of iron deficiency in the erythroid marrow. This is reflected by decreased serum iron and

Table 1. Laboratory findings in absolute iron deficiency

Stage 1: iron depletion
- Ferritin <12 µg/l

Stage 2: iron-deficient erythropoiesis
- Serum iron <60 µg/dl
- Transferrin saturation <20%
- Hypochromic RBC >5%
- CHr <26 pg
- Soluble transferrin receptor >7 mg/l
- Erythrocyte protoporphyrin >70 µg/dl

Stage 3: iron deficiency anaemia
- Haemoglobin <12 g/dl (female) or <13.5 g/dl (male)
- Haematocrit <36% (female) or <41% (male)
- RBC number normal then decreased
- MCV <80 fl (microcytosis)
- MCH <28 pg (hypochromia)

transferrin saturation (demonstrating an imbalance between iron needs and iron supply) and increased serum transferrin. This translates into signs of iron deficiency in erythroid precursors, including increased red cell protoporphyrin, soluble transferrin receptor, as well as decreased CHr and elevated percentage of hypochromic red blood cells. Iron deficiency anaemia, characterised by decreased haemoglobin and Hct with microcytosis (low MCV) and hypochromia (low MCH), is thus a late sign of advanced iron deficiency (stage 3). The number of red blood cells first remains normal but finally also decreases as the anaemia worsens.

Functional iron deficiency

This standard definition of iron deficiency by decreased serum ferritin levels and the classical separation into these three stages is, however, challenged by new data. Indeed, iron-deficient erythropoiesis and anaemia may occur even if iron stores are not exhausted or are even elevated [95,96]. This is called functional iron deficiency, defined as an imbalance between iron needs in the bone marrow and iron supply by macrophages (Table 2 and Figure 3). This can be encountered in two different situations, corresponding to either increased iron needs or decreased iron supply. Iron needs are increased when marrow erythroid activity is stimulated, the best example being provided by rHuEpo therapy [95,96]. Macrophage iron, originating from normal or even elevated stores as well as from haemoglobin iron recycled when senescent red blood cells are phagocytosed, may not be mobilised with sufficient speed to match iron needs for the production of new red cells. Therefore, serum iron and transferrin saturation decrease and other signs of iron-deficient erythropoiesis appear. Functional iron deficiency also develops when iron release by macrophages is

Table 2. Laboratory findings in functional iron deficiency

Normal or increased ferritin

Laboratory signs of iron deficient erythropoiesis:

- Serum iron <60 µg/dl
- Transferrin saturation <20%
- Hypochromic RBC >5%
- CHr <26 pg
- Soluble transferrin receptor >7 mg/l
- Erythrocyte protoporphyrin >70 µg/dl

Possible signs of functional iron deficiency anaemia:

- Haemoglobin <12 g/dl (female) or <13.5 g/dl (male)
- Haematocrit <36% (female) or <41% (male)
- RBC number normal then decreased
- MCV <80 fl (microcytosis)
- MCH <28 pg (hypochromia)

impaired [1–3,42,43,45]. A typical situation corresponding to this pattern is inflammation, due to infection, cancer or inflammatory disorders: iron is sequestered within macrophages, resulting in elevated serum ferritin values, while the erythron lacks iron, as indicated by increased red cell protoporphyrin, sTfR, as well as decreased CHr and an elevated percentage of hypochromic red blood cells. This may then translate into anaemia whose characteristics are identical to those of true iron deficiency, but for serum ferritin, which remains normal or elevated.

Iron deficiency during treatment with erythropoietic agents

Factors influencing response to erythropoietic agents

A number of factors may interfere with response to erythropoietic agents in cancer patients, reflecting differences in disease- and treatment-related factors, but also large variations in dose, frequency and route of administration, duration of therapy and the response criteria used [10–12]. These include red cell loss resulting from hypersplenism, haemolysis, haemorrhage or iatrogenic phlebotomies. Red cell production may be diminished by bone marrow infiltration, marrow necrosis, haemophagocytosis, myelofibrosis, deficiency of erythropoietic cofactors (folic acid, vitamin B12, iron) or infections. However, the type of tumour and moderate marrow involvement have generally not influenced the response rate. Patients who have been heavily pretreated with chemotherapy usually experience severe stem cell damage that should considerably interfere with response to erythropoietic agents. For patients treated concomitantly with chemotherapy, there is no marked difference between those receiving platinum-based regimens and those receiving other forms of chemotherapy. Patients receiving chemotherapy of moderate intensity respond as well as those not receiving concomitant chemotherapy, but the more intensive chemotherapy regimens are associated with lower response rates. Finally, complications of chemotherapy, such as inflammation, infections, nutritional deficiencies or bleeding, may have a negative impact upon response. All these factors are reviewed in other chapters.

Functional iron deficiency

Functional iron deficiency is a major factor limiting the efficacy of erythropoietic agents (Table 2). It is defined as an iron deficit in the functional erythroid compartment, the result of an imbalance between iron needs in the erythroid marrow and iron supply (Figure 3). This may occur even in the presence of large iron stores when storage iron release is inadequate. Iron requirements are determined by the overall level of erythropoietic activity and iron availability depends on the level of iron stores and their rate of mobilisation. Functional

iron deficiency can occur before erythropoietin therapy is started, either because iron stores are absent (true iron deficiency) or because storage iron release is impaired, a typical feature of the anaemia of chronic disorders [33]. It can also develop in the course of erythropoietin therapy when iron stores become progressively exhausted or more frequently when the increased iron needs of an expanding erythroid marrow cannot be matched by sufficient mobilisation of often enlarged iron stores. Iron-deficient erythropoiesis has been clearly identified in iron-replete subjects during red blood cell expansion by rHuEpo [95,96]. Indeed, the vast majority of renal failure patients treated with rHuEpo develop functional iron deficiency that seriously limits their erythropoietic response [98]. Similar observations have been made in patients receiving rHuEpo to facilitate an aggressive programme of autologous blood donation [99]. Although this has not been specifically examined in cancer patients treated with erythropoietic agents, there is every reason to believe that its prevalence is also very high in this setting.

A predictive algorithm of response to rHuEpo has first been proposed in the setting of anaemia associated with renal failure [100]. The best prediction by baseline parameters only was obtained with pretreatment soluble transferrin receptor (sTfR) and fibrinogen. There was 100% response rate when both sTfR and fibrinogen were low, vs only 29% when they were both high, and 67% when one was low and the other high. Changes of sTfR after 2 weeks of treatment were also predictive. When the 2-week sTfR increment was ≥20%, the response rate was 96%. When sTfR increment was <20 %, the response rate was 100% when baseline sTfR was low and fibrinogen normal, 12% when baseline fibrinogen was elevated and 62% when baseline fibrinogen was normal but baseline sTfR high. These prognostic factors illustrate the importance of the early erythropoietic response (changes of sTfR levels), subclinical inflammation (fibrinogen) and functional iron deficiency (baseline sTfR).

Functional iron deficiency is best diagnosed by a percentage of reticulocytes with a haemoglobin content lower than 23 pg [91,95] or a percentage of hypochromic red cells greater than 10% [101], with both parameters calculated by some automated haematologic cell counters. Alternatively, it can also be suspected when transferrin saturation falls below 20%. On the other hand, serum ferritin is of very limited value because it only gives an evaluation of iron stores without providing any hint of how these stores can be mobilised [102].

Iron supplementation

There is some concern that tumour cells may need iron for optimal growth [60], therefore routine iron supplementation of all cancer patients receiving erythropoietic agents is not recommended. The same is true for oral as well as intravenous iron supplementation. However, this should be balanced with the fact that transfusion of one red blood cell unit also provides a large amount (200 mg) of iron. Iron supplements should be given when absolute iron deficiency is

suspected, i.e., when serum ferritin is below 40–100 µg/l, a level associated with absence of iron stores in the anaemia of chronic disorders. Otherwise, iron supplements should be given when the transferrin saturation is below 20%, or the percentage of hypochromic red cells greater than 10%, and may be discontinued when the patient stabilises within the normal range.

The experience in iron-replete dialysis patients [103–106] has clearly indicated that oral iron supplementation is only marginally superior to no iron [14] but that intravenous iron both substantially improves response when erythropoietin therapy is instituted [14] and allows considerable (of the order of 40%) reduction in rHuEpo dose requirements during the maintenance phase [15,16,107–109]. Iron sucrose and iron gluconate are as effective for this purpose [110]. In predialysis patients the i.v. route of iron supplementation has also proved superior to the oral route [111]. Sufficient doses should be given because low doses of i.v. iron have not been as effective in such patients [112]. Several guidelines have been published to provide treatment schedules with i.v. iron in these patients [113–115]. An initial weekly loading dose of 100–300 mg i.v. iron is recommended during the correction phase of the anaemia, while much lower doses are necessary during the maintenance phase. The target serum ferritin level is in the range of 200–500 µg/l, that of hypochromic red cells below 2.5% and that of transferrin saturation 25–40%. To avoid toxicity from iron excess, i.v. iron should be withheld when transferrin saturation is above 50% and/or serum ferritin greater than 1000 µg/l. Oral iron should only be given to patients in whom i.v. supplements are not feasible or who do not tolerate them. On the other hand, low-dose intravenous iron has not been shown to be superior to energetic oral iron supplementation in patients pursuing a programme of autologous blood donation [116]. However, i.v. iron was superior to oral iron in patients undergoing preoperative stimulation of erythropoiesis without blood collection [117], but this was no longer the case when lower doses of i.v. iron were used [118].

Provided that the guidelines are respected, the safety of systematic i.v. iron supplementation has been demonstrated in renal failure patients undergoing treatment with erythropoietic agents [106,119–122]. There are three major forms of intravenous iron on the market (iron dextran, iron gluconate and iron sucrose or saccharate) whose availability varies from country to country. All consist of complexes of ferric iron surrounded by molecules ensuring its stability in plasma. After i.v. injection, all three compounds are primarily taken up by cells of the reticuloendothelial system (Figure 4). There iron is liberated from the complex and released to plasma transferrin that can then transport it to the erythroid marrow. If too much iron is released too fast, plasma transferrin will become rapidly oversaturated and NTBI will appear with all its potential toxicities. However, the three compounds are processed by the macrophages at very different speeds. Iron is very slowly released over a period of weeks from iron dextran complexes, allowing injection of very large doses of iron in a single infusion ("total dose infusion"). Iron is much more rapidly liberated from iron sucrose and iron gluconate complexes, so that their maximum tolerated doses

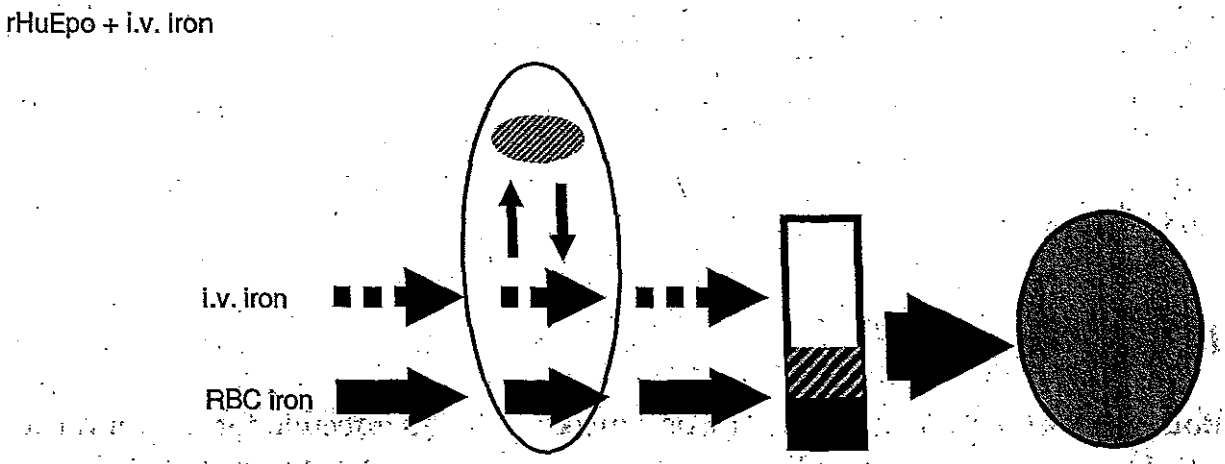

Fig. 4. Correction of functional iron deficiency by intravenous iron. The plain arrows represent recycling of red blood cell iron, as described in the third panel of Figure 3 "Erythropoietin therapy" in which the expansion of erythroid marrow by erythropoietin causes functional iron deficiency. The additional iron provided by intravenous iron products (dotted line) is also first taken up by macrophages that process it to release iron from the iron-glycan complex. Iron is then available for release by macrophages to plasma transferrin. In combination with the iron recycled from phagocytosed red cells, this allows for correction of transferrin saturation (hatched area) and provision of sufficient iron for erythropoiesis. The erythroid marrow can further expand without limitation by the iron supply.

are limited to approximately 400 mg and 100 mg iron, respectively. On the other hand, iron dextran has been associated with rare but potentially fatal anaphylactic reactions, and has been withdrawn from the market in many countries for this reason. The safety profile of iron sucrose [123] or iron gluconate [124] makes them the preferred intravenous compounds [113]. Iron sucrose has the advantage of allowing higher iron doses to be given at once, because iron gluconate at comparable doses would be associated with greater toxicity due to free iron release.

Iron usage has not been energetically pursued in clinical trials of erythropoietic agents in cancer patients and was generally left to the discretion of the individual investigator [13]. This was based on the false perception that cancer patients do not really need iron together with erythropoietin because their iron stores (ferritin) are not decreased. In addition, iron has usually been given orally, a method proving to be of little efficacy in renal failure patients and presumably even less effective in cancer patients because of impaired iron absorption, another characteristic of the anaemia of chronic disorders [1,44]. The safety and efficacy of intravenous iron to correct functional iron deficiency and improve anaemia has been well documented in rheumatoid arthritis during rHuEpo therapy [125] or after failure of oral iron in juvenile chronic arthritis, another form of anaemia of chronic disorder [126]. Apart from anecdotal reports on the efficacy of i.v. iron in patients failing to respond to erythropoietic agents, iron supplementation has not been formally studied in the anaemia of cancer.

Based on the experience in renal failure patients, intravenous administration of 100–300 mg elemental iron every week or every other week during the correction phase of anaemia will ensure the best utilisation of any given dose of rHuEpo. Ongoing clinical trials are investigating the safety and efficacy of intravenous iron in cancer patients treated with erythropoietic agents to demonstrate greater efficacy and/or lower erythropoietin requirements.

In practice

Routine iron supplementation of all cancer patients receiving erythropoietic agents is not recommended. In addition, no guidelines have been developed specifically for cancer patients and all recommendations can only be based on the experience in renal failure patients. The indications for iron supplementation include absolute iron deficiency (serum ferritin below 40–100 µg/l in cancer patients) and functional iron deficiency. The latter can be diagnosed by either a transferrin saturation below 20%, a percentage of hypochromic red cells above 10% or a CHr below 23 pg, even in the presence of adequate storage iron (normal or increased ferritin).

Intravenous administration of 100–300 mg elemental iron every week or every other week during the correction phase of anaemia will ensure the best utilisation of erythropoietic agents. Iron should be continued until transferrin saturation stabilises between 25 and 40% and the percentage of hypochromic red cells is below 2.5%. To avoid toxicity from iron excess, i.v. iron should be withheld when transferrin saturation is above 50% and/or serum ferritin greater than 1000 µg/l.

A safe, easy and often adequate schedule is 200 mg of iron sucrose (in 200 ml saline over 1 hour) per week for a total of three doses or two doses of 300 mg (in 250 ml saline over 90 minutes) given 2 weeks apart. Concomitant administration with chemotherapy should be avoided because transiently elevated transferrin saturation may enhance the toxicity of some chemotherapeutic agents.

References

1 Means RT Jr, Krantz SB. Progress in understanding the pathogenesis of the anemia of chronic disease. Blood 1992; 80: 1639–47
2 Beguin Y. Erythropoietin and the anemia of cancer. Acta Clin Belg 1996; 51: 36–52
3 Moliterno AR, Spivak JL. Anemia of cancer. Hematol Oncol Clin North Am 1996; 10: 345–63
4 Groopman JE, Itri LM. Chemotherapy-induced anemia in adults: incidence and treatment. J Natl Cancer Inst 1999; 91: 1616–34
5 Turner R, Anglin P, Burkes R et al. Epoetin alfa in cancer patients: evidence-based guidelines. J Pain Symptom Manage 2001; 22: 954–65
6 Seidenfeld J, Piper M, Flamm C et al. Epoetin treatment of anemia associated with cancer therapy: a systematic review and meta-analysis of controlled clinical trials. J Natl Cancer Inst 2001; 93: 1204–14

7 Seidenfeld J, Piper M, Aronson N. Systematic review of controlled trials on erythropoietin to support evidence-based guidelines. Oncology (Huntingt) 2002; 16: 171–88
8 Beguin Y. A risk–benefit assessment of epoetin in the management of anaemia associated with cancer. Drug Safety 1998; 19: 269–82
9 Beguin Y. Prediction of response to optimize outcome of treatment with erythropoietin. Semin Oncol 1998; 25 (Suppl 7): 27–34
10 Beguin Y. Prediction of response to rHuEpo. In: Bokemeyer C, Ludwig H, eds. Anaemia of Cancer. Amsterdam: Elsevier 2001; 153–65
11 Beguin Y. Predictive factors for response of anemia to recombinant human erythropoietin. In: Nowrousian MR, ed. Recombinant Human Erythropoietin (rhEpo) in Clinical Oncology. Berlin-Heidelberg: Springer-Verlag 2002; 263–86
12 Beguin Y. Prediction of response and other improvements on the limitations of recombinant human erythropoietin therapy in anemic cancer patients. Haematologica 2002; 87: 1209–21
13 Glaspy J, Cavill I. Role of iron in optimizing responses of anemic cancer patients to erythropoietin. Oncology (Huntingt) 1999; 13: 461–73
14 Macdougall IC, Tucker B, Thompson J, Tomson CR, Baker LR, Raine AE. A randomized controlled study of iron supplementation in patients treated with erythropoietin. Kidney Int 1996; 50: 1694–9
15 Fishbane S, Frei GL, Maesaka J. Reduction in recombinant human erythropoietin doses by the use of chronic intravenous iron supplementation. Am J Kidney Dis 1995; 26: 41–6
16 Besarab A, Amin N, Ahsan M et al. Optimization of epoetin therapy with intravenous iron therapy in hemodialysis patients. J Am Soc Nephrol 2000; 11: 530–8
17 Goodnough LT, Skikne B, Brugnara C. Erythropoietin, iron, and erythropoiesis. Blood 2000; 96: 823–33
18 Testa U. Recent developments in the understanding of iron metabolism. Hematol J 2002; 3: 63–89
19 Andrews NC. Disorders of iron metabolism. N Engl J Med 1999; 341: 1986–95
20 Andrews NC. Iron homeostasis: insights from genetics and animal models. Nat Rev Genet 2000; 1: 208–17.
21 Aisen P, Enns C, Wessling-Resnick M. Chemistry and biology of eukaryotic iron metabolism. Int J Biochem Cell Biol 2001; 33: 940–59
22 Conrad ME, Umbreit JN. Iron absorption and transport – an update. Am J Hematol 2000; 64: 287–98
23 Roy CN, Enns CA. Iron homeostasis: new tales from the crypt. Blood 2000; 96: 4020–7
24 Morgan EH, Oates PS. Mechanisms and regulation of intestinal iron absorption. Blood Cells Mol Dis 2002; 29: 384–99
25 Chorney MJ, Yoshida Y, Meyer PN, Yoshida M, Gerhard GS. The enigmatic role of the hemochromatosis protein (HFE) in iron absorption. Trends Mol Med 2003; 9: 118–25
26 Nicolas G, Viatte L, Bennoun M, Beaumont C, Kahn A, Vaulont S. Hepcidin, a new iron regulatory peptide. Blood Cells Mol Dis 2002; 29: 327–35
27 Wessling-Resnick M. A possible link between hepcidin and regulation of dietary iron absorption. Nutr Rev 2002; 60: 371–4
28 Ponka P, Lok CN. The transferrin receptor: role in health and disease. Int J Biochem Cell Biol 1999; 31: 1111–37
29 Haile DJ. Regulation of genes of iron metabolism by the iron-response proteins. Am J Med Sci 1999; 318: 230–40
30 Lieu PT, Heiskala M, Peterson PA, Yang Y. The roles of iron in health and disease. Mol Aspects Med 2001; 22: 1–87
31 Beguin Y. Soluble transferrin receptor for the evaluation of erythropoiesis and iron status. Clin Chim Acta 2003; 329: 9–22

32 Beguin Y, Stray SM, Cazzola M, Huebers HA, Finch CA. Ferrokinetic measurement of erythropoiesis. Acta Haematol 1988; 79: 121–6
33 Fillet G, Beguin Y, Baldelli L. Model of reticuloendothelial iron metabolism in humans: abnormal behavior in idiopathic hemochromatosis and in inflammation. Blood 1989; 74: 844–51
34 Torti FM, Torti SV. Regulation of ferritin genes and protein. Blood 2002; 99: 3505–16
35 Weiss G, Wachter H, Fuchs D. Linkage of cell-mediated immunity to iron metabolism. Immunol Today 1995; 16: 495–500
36 Weiss G. Iron and anemia of chronic disease. Kidney Int (Suppl) 1999; 69: S12–7
37 Domachowske JB, Rafferty SP, Singhania N, Mardiney M, Malech HL. Nitric oxide alters the expression of gamma-globin, H-ferritin, and transferrin receptor in human K562 cells at the posttranscriptional level. Blood 1996; 88: 2980–8
38 Spivak JL. Cancer-related anemia: its causes and characteristics. Semin Oncol 1994; 21 (Suppl 3): 3–8
39 DeGowin RL, Gibson DP. Erythropoietin and the anemia of mice bearing extramedullary tumor. J Lab Clin Med 1979; 94: 303–11
40 Doll DC, Weiss RB. Neoplasia and the erythron. J Clin Oncol 1985; 3: 429–46
41 Cavill I, Bentley DP. Erythropoiesis in the anaemia of rheumatoid arthritis. Br J Haematol 1982; 50: 583–90
42 Means RT Jr. Advances in the anemia of chronic disease. Int J Hematol 1999; 70: 7–12
43 Weiss G. Pathogenesis and treatment of anaemia of chronic disease. Blood Rev 2002; 16: 87–96
44 Sears D. Anemia of chronic disease. Med Clin North Am 1992; 76: 567–79
45 Means RT Jr. Pathogenesis of the anemia of chronic disease: a cytokine-mediated anemia. Stem Cells 1995; 13: 32–7
46 Rodriguez-Cimadevilla JC, Beauchemin V, Villeneuve L, Letendre F, Shaw A, Hoang T. Coordinate secretion of interleukin-1 beta and granulocyte-macrophage colony-stimulating factor by the blast cells of acute myeloblastic leukemia: role of interleukin-1 as an endogenous inducer. Blood 1990; 76: 1481–9
47 Foa R, Massaia M, Cardona S et al. Production of tumor necrosis factor-alpha by B-cell chronic lymphocytic leukemia cells: a possible regulatory role of TNF in the progression of the disease. Blood 1990; 76: 393–400
48 Naumovski L, Utz PJ, Bergstrom SK et al. SUP-HD1: a new Hodgkin's disease-derived cell line with lymphoid features produces interferon-gamma. Blood 1989; 74: 2733–42
49 Kremer JP, Reisbach G, Nerl C, Dormer P. B-cell chronic lymphocytic leukaemia cells express and release transforming growth factor-beta. Br J Haematol 1992; 80: 480–7
50 Emilie D, Coumbaras J, Raphael M et al. Interleukin-6 production in high-grade B lymphomas: correlation with the presence of malignant immunoblasts in acquired immunodeficiency syndrome and in human immunodeficiency virus-seronegative patients. Blood 1992; 80: 498–504
51 Balkwill F, Osborne R, Burke F et al. Evidence for tumour necrosis factor/cachectin production in cancer. Lancet 1987; 2: 1229–32
52 Hollen CW, Henthorn J, Koziol JA, Burstein SA. Elevated serum interleukin-6 levels in patients with reactive thrombocytosis. Br J Haematol 1991; 79: 286–90
53 Torti FM, Torti SV. Cytokines, iron homeostasis, and cancer. Adv Exp Med Biol 1994; 354: 161–70
54 Konijn AM. Iron metabolism in inflammation. Clin Haematol 1994; 7: 829–49
55 Torti SV, Torti FM. Iron and ferritin in inflammation and cancer. Adv Inorg Biochem 1994; 10: 119–37
56 Richardson DR, Ponka P. The molecular mechanisms of the metabolism and transport of iron in normal and neoplastic cells. Biochim Biophys Acta 1997; 1331: 1–40

57 Cazzola M, Bergamaschi G, Dezza L, Arosio P. Manipulations of cellular iron metabolism for modulating normal and malignant cell proliferation: achievements and prospects. Blood 1990; 75: 1903–19
58 Kwok JC, Richardson DR. The iron metabolism of neoplastic cells: alterations that facilitate proliferation? Crit Rev Oncol Hematol 2002; 42: 65–78
59 Kontoghiorghes GJ, Weinberg ED. Iron: mammalian defense systems, mechanisms of disease, and chelation therapy approaches. Blood Rev 1995; 9: 33–45
60 Weinberg ED. The role of iron in cancer. Eur J Cancer Prev 1996; 5: 19–36
61 Hastka J, Lasserre JJ, Schwarzbeck A, Strauch M, Hehlmann R. Zinc protoporphyrin in anemia of chronic disorders. Blood 1993; 81: 1200–4
62 Ferguson BJ, Skikne BS, Simpson KM, Baynes RD, Cook JD. Serum transferrin receptor distinguishes the anemia of chronic disease from iron deficiency anemia. J Lab Clin Med 1992; 119: 385–90
63 Haurani FI, Green D, Young K. Iron absorption in hypoferremia. Am J Med Sci 1965; 249: 537–47
64 Haurani FI, Young K, Tocantins LM. Reutilization of iron in anemia complicating malignant neoplasms. Blood 1963; 22: 73–81
65 Zarrabi MH, Lysik R, DiStefano J, Zucker S. The anaemia of chronic disorders: studies of iron reutilization in the anaemia of experimental malignancy and chronic inflammation. Br J Haematol 1977; 35: 647–58
66 Temple JJ, Stuckey WJ. Mechanisms contributing to the anemia associated with a localized solid tumor. Am J Med Sci 1986; 292: 277–81
67 Douglas SW, Adamson JW. The anemia of chronic disorders: studies of marrow regulation and iron metabolism. Blood 1975; 45: 55–65
68 Gordeuk VR, Prithviraj P, Dolinar T, Brittenham GM. Interleukin 1 administration in mice produces hypoferremia despite neutropenia. J Clin Invest 1988; 82: 1934–8
69 Goldblum SE, Cohen DA, Jay M, McClain CJ. Interleukin 1-induced depression of iron and zinc: role of granulocytes and lactoferrin. Am J Physiol 1987; 252: E27–E32
70 Uchida T, Yamagiwa A, Nakamura K. The effect of interleukin-1 on iron metabolism in rats. Eur J Haematol 1991; 46: 1–5
71 Alvarez-Hernandez X, Liceaga J, McKay IC, Brock JH. Induction of hypoferremia and modulation of macrophage iron metabolism by tumor necrosis factor. Lab Invest 1989; 61: 319–22
72 Moldawer LL, Marano MA, Wei H et al. Cachectin/tumor necrosis factor-alpha alters red blood cell kinetics and induces anemia in vivo. FASEB J 1989; 3: 1637–43
73 Nieken J, Mulder NH, Buter J et al. Recombinant human interleukin-6 induces a rapid and reversible anemia in cancer patients. Blood 1995; 86: 900–5
74 Kobune M, Kohgo Y, Kato J, Miyazaki E, Niitsu Y. Interleukin-6 enhances hepatic transferrin uptake and ferritin expression in rats. Hepatology 1994; 19: 1468–75
75 Konijn AM, Hershko C. Ferritin synthesis in inflammation I. Pathogenesis of impaired iron release. Br J Haematol 1977; 37: 7–16
76 Konijn AM, Carmel N, Levy R, Hershko C. Ferritin synthesis in inflammation. Br J Haematol 1981; 49: 361–70
77 Rogers J, Lacroix L, Durmowitz G, Kasschau K, Andriotakis J, Bridges KR. The role of cytokines in the regulation of ferritin expression. Adv Exp Med Biol 1994; 356: 127–32
78 Rogers JT, Bridges KR, Durmowicz GP, Glass J, Auron PE, Munro HN. Translational control during the acute phase response. Ferritin synthesis in response to interleukin-1. J Biol Chem 1990; 265: 14572–8
79 Miller LL, Miller SC, Torti SV, Tsuji Y, Torti FM. Iron-independent induction of ferritin H chain by tumor necrosis factor. Proc Natl Acad Sci USA 1991; 88: 4946–50
80 Fahmy M, Young SP. Modulation of iron metabolism in monocyte cell line U937 by inflammatory cytokines: changes in transferrin uptake, iron handling and ferritin mRNA. Biochem J 1993; 296: 175–81

81 Scaccabarozzi A, Arosio P, Weiss G et al. Relationship between TNF-alpha and iron metabolism in differentiating human monocytic THP-1 cells. Br J Haematol 2000; 110: 978–84
82 Kim S, Ponka P. Effects of interferon-gamma and lipopolysaccharide on macrophage iron metabolism are mediated by nitric oxide-induced degradation of iron regulatory protein 2. J Biol Chem 2000; 275: 6220–6
83 Mulero V, Brock JH. Regulation of iron metabolism in murine J774 macrophages: role of nitric oxide-dependent and -independent pathways following activation with gamma interferon and lipopolysaccharide. Blood 1999; 94: 2383–9
84 Ryu SY, Jeong KS, Kang BN et al. Modulation of transferrin synthesis, transferrin receptor expression, iNOS expression and NO production in mouse macrophages by cytokines, either alone or in combination. Anticancer Res 2000; 20: 3331–8
85 Recalcati S, Taramelli D, Conte D, Cairo G. Nitric oxide-mediated induction of ferritin synthesis in J774 macrophages by inflammatory cytokines: role of selective iron regulatory protein-2 downregulation. Blood 1998; 91: 1059–66
86 Ponka P, Beaumont C, Richardson DR. Function and regulation of transferrin and ferritin. Semin Hematol 1998; 35: 35–54
87 Worwood M. Ferritin. Blood Rev 1990; 4: 259–69
88 Skikne BS, Flowers CH, Cook JD. Serum transferrin receptor: a quantitative measure of tissue iron deficiency. Blood 1990; 75: 1870–6
89 Suominen P, Punnonen K, Rajamaki A, Irjala K. Serum transferrin receptor and transferrin receptor-ferritin index identify healthy subjects with subclinical iron deficits. Blood 1998; 92: 2934–9
90 Punnonen K, Irjala K, Rajamaki A. Serum transferrin receptor and its ratio to serum ferritin in the diagnosis of iron deficiency. Blood 1997; 89: 1052–7
91 Brugnara C. Use of reticulocyte cellular indices in the diagnosis and treatment of hematological disorders. Int J Clin Lab Res 1998; 28: 1–11
92 d'Onofrio G, Chirillo R, Zini G, Caenaro G, Tommasi M, Micciulli G. Simultaneous measurement of reticulocyte and red blood cell indices in healthy subjects and patients with microcytic and macrocytic anemia. Blood 1995; 85: 818–23
93 Bain BJ, Cavill IA. Hypochromic macrocytes: are they reticulocytes? J Clin Pathol 1993; 46: 963–4
94 Mast AE, Blinder MA, Lu Q, Flax S, Dietzen DJ. Clinical utility of the reticulocyte hemoglobin content in the diagnosis of iron deficiency. Blood 2002; 99: 1489–91
95 Brugnara C, Colella GM, Cremins J et al. Effects of subcutaneous recombinant human erythropoietin in normal subjects: development of decreased reticulocyte hemoglobin content and iron-deficient erythropoiesis. J Lab Clin Med 1994; 123: 660–7
96 Brugnara C, Chambers LA, Malynn E, Goldberg MA, Kruskall MS. Red blood cell regeneration induced by subcutaneous recombinant erythropoietin: iron-deficient erythropoiesis in iron-replete subjects. Blood 1993; 81: 956–64
97 Provan D. Mechanisms and management of iron deficiency anaemia. Br J Haematol 1999; 105 (Suppl 1): 19–26
98 Macdougall IC. Strategies for iron supplementation: oral versus intravenous. Kidney Int (Suppl) 1999; 69: S61–6
99 Goodnough LT, Marcus RE. Erythropoiesis in patients stimulated with erythropoietin: the relevance of storage iron. Vox Sang 1998; 75: 128–33
100 Beguin Y, Loo M, R'Zik S et al. Early prediction of response to recombinant human erythropoietin in patients with the anemia of renal failure by serum transferrin receptor and fibrinogen. Blood 1993; 82: 2010–6
101 Macdougall IC, Cavill I, Hulme B et al. Detection of functional iron deficiency during erythropoietin treatment: a new approach. Br Med J 1992; 304: 225–6
102 Kooistra MP, van Es A, Struyvenberg A, Marx JJ. Iron metabolism in patients with the anaemia of end-stage renal disease during treatment with recombinant human erythropoietin. Br J Haematol 1991; 79: 634–9

103 Sunder-Plassmann G, Horl WH. Erythropoietin and iron [see comments]. Clin Nephrol 1997; 47: 141–57
104 Fishbane S, Maesaka JK. Iron management in end-stage renal disease [see comments]. Am J Kidney Dis 1997; 29: 319–33
105 Johnson DW, Herzig KA, Gissane R, Campbell SB, Hawley CM, Isbel NM. Oral versus intravenous iron supplementation in peritoneal dialysis patients. Perit Dial Int 2001; 21 (Suppl 3): S231–5
106 Agarwal R, Warnock D. Issues related to iron replacement in chronic kidney disease. Semin Nephrol 2002; 22: 479–87
107 Ahsan N. Intravenous infusion of total dose iron is superior to oral iron in treatment of anemia in peritoneal dialysis patients: a single center comparative study. J Am Soc Nephrol 1998; 9: 664–8
108 Ahsan N. Infusion of total dose iron versus oral iron supplementation in ambulatory peritoneal dialysis patients: a prospective, cross-over trial. Adv Perit Dial 2000; 16: 80–4
109 Johnson DW, Herzig KA, Gissane R, Campbell SB, Hawley CM, Isbel NM. A prospective crossover trial comparing intermittent intravenous and continuous oral iron supplements in peritoneal dialysis patients. Nephrol Dial Transplant 2001; 16: 1879–84
110 Kosch M, Bahner U, Bettger H, Matzkies F, Teschner M, Schaefer RM. A randomized, controlled parallel-group trial on efficacy and safety of iron sucrose (Venofer) vs iron gluconate (Ferrlecit) in haemodialysis patients treated with rHuEpo. Nephrol Dial Transplant 2001; 16: 1239–44
111 Aggarwal HK, Nand N, Singh S, Singh M, Hemant, Kaushik G. Comparison of oral versus intravenous iron therapy in predialysis patients of chronic renal failure receiving recombinant human erythropoietin. J Assoc Physicians India 2003; 51: 170–4
112 Stoves J, Inglis H, Newstead CG. A randomized study of oral vs intravenous iron supplementation in patients with progressive renal insufficiency treated with erythropoietin. Nephrol Dial Transplant 2001; 16: 967–74
113 Drueke TB, Barany P, Cazzola M et al. Management of iron deficiency in renal anemia: guidelines for the optimal therapeutic approach in erythropoietin-treated patients. Clin Nephrol 1997; 48: 1–8
114 Macdougall IC, Horl WH, Jacobs C et al. European best practice guidelines 6–8: assessing and optimizing iron stores. Nephrol Dial Transplant 2000; 15 (Suppl. 4): 20–32
115 NKF-DOQI clinical practice guidelines for the treatment of anemia of chronic renal failure. National Kidney Foundation – Dialysis Outcomes Quality Initiative [see comments]. Am J Kidney Dis 1997; 30: S192–240
116 Kasper SM, Lazansky H, Stark C, Klimek M, Laubinger R, Borner U. Efficacy of oral iron supplementation is not enhanced by additional intravenous iron during autologous blood donation. Transfusion 1998; 38: 764–70
117 Rohling RG, Zimmermann AP, Breymann C. Intravenous versus oral iron supplementation for preoperative stimulation of hemoglobin synthesis using recombinant human erythropoietin. J Hematother Stem Cell Res 2000; 9: 497–500
118 Olijhoek G, Megens JG, Musto P et al. Role of oral versus IV iron supplementation in the erythropoietic response to rHuEPO: a randomized, placebo-controlled trial. Transfusion 2001; 41: 957–63
119 Sunder-Plassmann G, Horl WH. Safety aspects of parenteral iron in patients with end-stage renal disease. Drug Safety 1997; 17: 241–50
120 Besarab A, Frinak S, Yee J. An indistinct balance: the safety and efficacy of parenteral iron therapy. J Am Soc Nephrol 1999; 10: 2029–43
121 Fishbane S, Kowalski EA. The comparative safety of intravenous iron dextran, iron saccharate, and sodium ferric gluconate. Semin Dial 2000; 13: 381–4

122 Fishbane S. Safety in iron management. Am J Kidney Dis 2003; 41: 18–26
123 Yee J, Besarab A. Iron sucrose: the oldest iron therapy becomes new. Am J Kidney Dis 2002; 40: 1111–21
124 Fishbane S, Wagner J. Sodium ferric gluconate complex in the treatment of iron deficiency for patients on dialysis. Am J Kidney Dis 2001; 37: 879–83
125 Vreugdenhil G, Manger B, Nieuwenhuizen C, Feelders RA, van Eijk HG, Swaak AJ. Iron stores and serum transferrin receptor levels during recombinant human erythropoietin treatment of anemia in rheumatoid arthritis. Ann Hematol 1992; 65: 265–8
126 Martini A, Ravelli A, Di Fuccia G, Rosti V, Cazzola M, Barosi G. Intravenous iron therapy for severe anaemia in systemic-onset juvenile chronic arthritis. Lancet 1994; 344: 1052–4