bjh review

# Diagnosis and management of anaemia of chronic disease: current status

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## Summary

Anaemia of chronic disease is the second most common form of anaemia worldwide, and is seen in a variety of inflammatory, infective and malignant diseases. Functional iron deficiency is fundamental to the pathogenesis of the anaemia, and the polypeptide, hepcidin, plays a key role. Diagnosis may be difficult, but new automated red cell indices, algorithms for detection of functional iron deficiency, and assays for hepcidin levels are being developed. Management of the causative disease process will usually improve haemoglobin levels, but where this is not possible, erythropoietic stimulating agents are often used, although there are still concerns about potential adverse effects, especially thromboembolism. There is increasing evidence that supplemental iron given parenterally can safely overcome the functional iron deficiency. Inhibitors of hepcidin, and various inflammatory modulators show promise for the future.

**Keywords:** anaemia of chronic disease, inflammation, hepcidin, iron, erythropoietin.

Anaemia of chronic disease (ACD), or anaemia of inflammation, is the term used to describe the hypoproliferative anaemia seen in response to systemic illness or inflammation. It is the second most prevalent form of anaemia after iron deficiency anaemia (IDA) and the most common amongst patients with chronic illnesses (Dallman *et al*, 1984). It is seen in a variety of conditions, including infections, cancer and autoimmune conditions and is typically normochromic and normocytic, characterized by low serum iron, decreased transferrin saturation, decreased bone marrow sideroblasts and increased reticuloendothelial iron (Cartwright, 1966). The mechanisms that produce the anaemia include impaired production of erythropoietin (EPO), blunted marrow erythroid response to EPO, iron-restricted erythropoiesis, and a diminished pool of EPO-responsive cells. This article will briefly review the pathogenesis of ACD, and then discuss the diagnosis and current and future management strategies for the condition. The anaemia of chronic renal failure, although sometimes referred to as ACD, will not be discussed in this review, except where useful insights into other forms of ACD can be derived from relevant papers.

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## Causes and pathogenesis

Some of the conditions associated with ACD are listed in Table I. These diseases all share features of acute or chronic immune activation, and it is now understood that several immune mechanisms contribute to the anaemia observed in ACD. Similar patterns of anaemia are seen in other clinical situations without overt clinical inflammation, for example the anaemia of ageing (Ferrucci & Balducci, 2008) and the anaemia of cardiac failure (Opasich *et al*, 2005). It has been postulated that more subtle pro-inflammatory changes may account for the anaemia seen in some elderly patients. Cytokines and cells of the reticuloendothelial system stimulated by the inflammatory milieu produce changes in iron homeostasis, reduce EPO production and proliferation of erythroid progenitors, and shorten red cell lifespan.

### *Altered iron homeostasis*

Low serum iron levels are a common feature of ACD: mice injected with pro-inflammatory cytokines, interleukin-1 (IL-1) and tumour necrosis factor-alpha (TNF-α) developed hypoferraemia and anaemia (Alvarez-Hernández *et al*, 1989), and, in human volunteers, injection of interleukin-6 (IL-6) caused reduction in serum iron levels and transferrin saturation (Nemeth *et al*, 2004a), now known to be mediated via a 25 amino acid polypeptide hormone known as hepcidin (Park *et al*, 2001; Rivera *et al*, 2005).

Hepcidin is produced by hepatocytes (and to a lesser extent adipocytes and macrophages) and plays a key role in the regulation of iron balance and transport (reviewed by Nemeth & Ganz, 2009). The hormone's actions are mediated through its binding to ferroportin, the principal cellular iron efflux protein, resulting in the blockade of iron export from body iron stores in macrophages and hepatocytes; inhibition of iron absorption by duodenal enterocytes also occurs, although recent evidence suggests that this may be caused by downre-

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**Table I.** Diseases associated with ACD.

| Associated diseases |
|---|
| Infections |
| Viral |
| Bacterial |
| Parasitic |
| Fungal |
| Malignancies |
| Haematological |
| Solid tumours |
| Autoimmune |
| Rheumatoid arthritis |
| Systemic lupus erythematosus and related conditions |
| Vasculitis |
| Sarcoidosis |
| Inflammatory bowel disease |
| Renal |
| Chronic renal failure |
| Cardiac |
| Chronic heart failure |

gulation of another transport protein, divalent metal transporter-1 (DMT-1) rather than ferroportin (Brasse-Lagnel *et al*, 2011; Burpee *et al*, 2011). The combined effect is to restrict iron availability for erythropoiesis, sometimes referred to as a state of 'functional iron deficiency', and to result in iron accumulation in tissue macrophages (Fig 1). Hepcidin overexpression in transgenic mice reproduces many of the features of ACD (Roy *et al*, 2007), and hepcidin levels are raised in a variety of inflammatory disorders (Ganz *et al*, 2008; Sharma *et al*, 2008; Busbridge *et al*, 2009; Demirag *et al*, 2009; de Mast *et al*, 2009; Hohaus *et al*, 2010). Once bound to ferroportin, the ligand-receptor complex is internalized and degraded, and cellular iron export ceases (Nemeth *et al*, 2004b).

Regulation of hepcidin production occurs through recognition of iron levels and erythropoietic activity. Thus iron excess stimulates hepcidin production, leading to reduced iron absorption and switching off iron release from tissue stores. Conversely, in iron deficiency, hepcidin production is suppressed, enabling increased iron absorption and release of storage iron: similar changes occur when erythroid activity increases. In inflammatory conditions, hepcidin production is increased, and IL-6 has been shown to be a potent inducer of hepcidin via signal transducer and activator of transcription-3 (STAT-3) signalling (Nemeth *et al*, 2004a; Wrighting & Andrews, 2006). There is also evidence of a role for other inflammatory cytokines, including IL-1 (Lee *et al*, 2005), and bone morphogenetic proteins (BMPs) 2, 4, 6 and 9 (Truksa *et al*, 2006). Parallel processes can be seen in malignant conditions. For example, in patients with Hodgkin lymphoma, hepcidin levels were closely correlated with levels of IL-6, rather than other cytokines (Hohaus *et al*, 2010) whereas a recent study suggests that BMP-2, rather than IL-6, is the key inducer of hepcidin in patients with multiple myeloma: hepcidin levels in patients with myeloma inversely correlate with haemoglobin levels, and anti-BMP-2 antibodies blocked the hepcidin-inducing activity of sera from patients with myeloma more consistently than anti-IL-6 antibodies (Maes *et al*, 2010).

That the erythropoietic and inflammatory pathways regulating hepcidin production may be separate was suggested by a study by Theurl *et al* (2009): using a rat model of ACD, they demonstrated that animals with ACD rendered iron-deficient by phlebotomy had lower hepcidin levels than animals with ACD alone. Similar findings were noted in patients with ACD/IDA when compared to individuals with ACD, and the former were able to absorb dietary iron and mobilize iron from macrophage stores. This is an important observation if hepcidin levels are to be incorporated into the diagnostic pathway for patients with ACD.

### *Reduced EPO production*

Under normal physiological conditions, levels of EPO are inversely correlated with haemoglobin levels and tissue oxygenation, but in chronic inflammatory conditions the EPO response is blunted, leading to inadequate levels of EPO

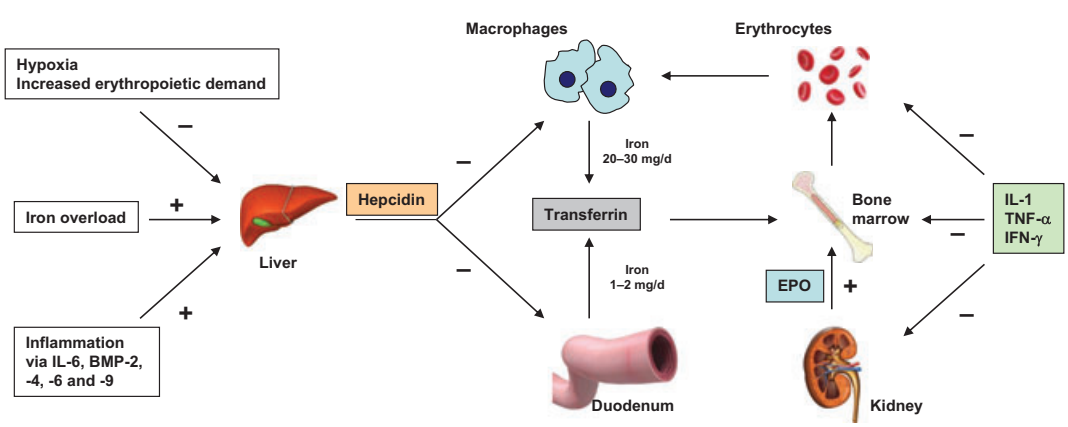


**Fig 1.** Effects of inflammation on erythropoiesis and iron metabolism. Key: + = stimulatory effect; − = inhibitory effect; for other abbreviations see text.


 

for the degree of anaemia (Miller *et al*, 1990), and this is thought to be mediated via inflammatory cytokines such as IL-1 and tumour necrosis factor-α (TNF-α) (Faquin *et al*, 1992). However, other studies have shown conflicting results, so blunted EPO response may not be universal in ACD (Cazzola *et al*, 1996).

### *Reduced erythroid responsiveness*

In ACD, the proliferation and differentiation of erythroid progenitor cells is reduced. Early studies showed that macrophages from patients with ACD would suppress erythroid colony formation *in vitro* (Zanjani *et al*, 1982). Subsequent studies showed this effect to be due to inhibitory effects of inflammatory cytokines, especially interferon-γ, on growth of erythroid burst-forming units (BFU-E) and erythroid colony-forming units (CFU-E) (Means, 1995; Chasis & Mohandas, 2008), and that this effect could be overcome by addition of high concentrations of EPO to the culture systems (Johnson *et al*, 1989). Hepcidin itself has an inhibitory effect on erythropoiesis *in vitro* at low EPO concentrations (Dallalio *et al*, 1996).

Papadaki *et al* (2002) demonstrated that bone marrow cultures from patients with active rheumatoid arthritis showed defective growth when compared to normal controls, and that there was an inverse correlation between colony growth and levels of TNF-α in the culture supernatant. Moreover, these effects were reversed both *in vitro* and *in vivo* following treatment with infliximab, an antibody against TNF-α.

### *Reduced red cell survival*

Early studies suggested that red cell survival is shortened in ACD (Cartwright, 1966) and more recent research using breath carbon monoxide levels to assess red cell survival confirmed that red cell survival is modestly shortened in patients with rheumatoid arthritis and anaemic hospital inpatients (Mitlyng *et al*, 2006) and may be a contributory factor in ACD, but there have been no direct studies of the mechanisms involved: these may include increased erythrophagocytosis induced by inflammatory cytokines (Beaumont & Canonne-Hergaux, 2005) and oxidative damage to erythrocytes, causing reduced survival.

## Diagnostic issues in ACD

The widespread settings in which ACD may be seen can make diagnosis difficult: thus confounding conditions, haemoglobinopathies, nutritional deficiencies, bleeding or haemolysis, medications, recurrent phlebotomy, bone marrow infection or infiltration may all affect the haematological picture (Vreugdenhil *et al*, 1990a). Typically the anaemia is mild to moderate (Weiss & Goodnough, 2005), normochromic and normocytic (although anaemia may become microcytic as disease progresses) and the reticulocyte count is low, reflecting the hypoproliferative nature of the anaemia. Inflammation may be inferred from other features of the blood count, such as neutrophilia, monocytosis or thrombocytosis, and through measurement of non-specific inflammatory markers, such as C-reactive protein (CRP) or erythrocyte sedimentation rate (ESR).

Exclusion of IDA is very important in the work-up of patients with ACD, although the two conditions frequently co-exist. Typically, serum iron and transferrin saturation are both decreased in ACD and iron deficiency, indicating limited iron supply to the erythron, but transferrin levels are increased in IDA, whereas in ACD they are normal or decreased. Measurement of serum ferritin is frequently of little value, as ferritin is an acute phase protein as well as an indicator of iron stores, and levels will be increased in the presence of inflammation. The gold standard for assessment of iron stores remains a Perl's stained bone marrow aspirate, but a bone marrow biopsy is otherwise of limited value in the diagnosis of ACD, so other non-invasive tools for measurement of iron supply are needed.

### *Serum transferrin receptor (sTFR) and sTFR/ferritin ratio*

The measurement of sTFR, the truncated fragment of the membrane receptor, has been suggested as a possible tool for differentiating between ACD and IDA. The transferrin receptor is found on virtually all cells in the body, but is present at high levels on erythroid progenitors. sTFR levels increase in IDA as the availability of iron for erythropoiesis decreases (Cook *et al*, 1993), whereas in ACD levels may not differ from steady state because transferrin receptor expression is negatively affected by inflammatory cytokines (Fitzsimons *et al*, 2002). However, in practice interpretation of this assay in differentiating IDA from ACD has proved more difficult (Mast *et al*, 1998), and the assay has not been standardized.

The ratio of sTFR to the log of the serum ferritin has been proposed to be a useful tool in the diagnosis of ACD, and particularly in differentiating ACD from IDA (Punnonen *et al*, 1997; Suominen *et al*, 2000; Malope *et al*, 2001; Skikne, 2008). A ratio <1 makes ACD likely, whereas ratios >2 suggest that iron stores are deficient, with or without ACD.

### *Red cell indices*

Many modern haematology analysers are capable of calculating new red cell indices that may be useful in the evaluation of different forms of anaemia. Two of these, the reticulocyte haemoglobin content (CHr) and the percentage hypochromic red cells (%HYPO) (reported by Bayer Advia 120 haematology analyser, Siemens Healthcare Diagnostics, Deerfield, IL, USA) can provide information about iron supply to the erythron, and may be useful in guiding the management of ACD. CHr is a measure of haemoglobin in the most recently formed erythrocytes, while the %HYPO indicates the percentage of cells with haemoglobin content of <280 g/l. The former gives a

relatively acute evaluation (48 h) of recent bone marrow activity, whereas the latter gives a time-averaged picture (20–120 d) (Goodnough *et al*, 2010). Similar indices can be reported by the Sysmex XE-2100 analyser (Sysmex, Mundelein, IL, USA), which derives RET-Y (equivalent to CHr) and RBC-Y (equivalent to HYPO%). CHr has been shown to be a useful tool in the detection of early iron deficiency, as well as in monitoring early response to iron therapy (Brugnara *et al*, 1994).

Thomas and Thomas (2002) studied the relationship between CHr, %HYPO and sTFR/ferritin ratio to evaluate anaemia in 442 patients with disease-specific anaemias and 154 non-anaemic subjects. A simple plot of CHr against sTFR/ferritin divided anaemic samples into four functional quadrants: (i) iron replete, normal eythropoiesis; (ii) reduced iron supply but not yet iron-deficient erythropoiesis; (iii) iron depleted with iron-deficient erythropoiesis; (iv) iron replete but with functional iron deficiency leading to decreased haemoglobinization. This may help in deciding whether iron supplementation may improve haemoglobin levels in individual patients.

### *Hepcidin assays*

The central role played by hepcidin in the pathogenesis of ACD suggests that measurement of hepcidin levels might be a useful diagnostic tool in the evaluation of possible ACD. The first tools for measurement of hepcidin relied on extraction from urine, and were laborious (Nemeth *et al*, 2004a), but more recently mass spectrometric (Kemna *et al*, 2007; Murphy *et al*, 2007) and immunological methods (Ganz *et al*, 2008; Busbridge *et al*, 2009) to measure hepcidin levels in urine and serum have been developed with potential for clinical use, and some are now commercially available. Elevated serum hepcidin levels have been observed in a variety of inflammatory diseases, including rheumatological conditions, inflammatory bowel disease, infections, multiple myeloma, non-Hodgkin lymphoma and critical illness (Ganz *et al*, 2008; Sharma *et al*, 2008; Busbridge *et al*, 2009; Demirag *et al*, 2009; de Mast *et al*, 2009; Hohaus *et al*, 2010). However, whilst hepcidin levels will usually be elevated in these inflammatory anaemias, levels may not be elevated in patients who have co-existent ACD/IDA as the inflammation-induced increase in hepcidin production will be opposed by the effects of iron deficiency: indeed the long-term effects of hepcidin may to be produce iron deficiency through reduced intestinal iron absorption, so the use of hepcidin levels to diagnose ACD will need to be evaluated carefully (Goodnough *et al*, 2010). Hepcidin levels may therefore be more useful in distinguishing patients with pure ACD from those with combined ACD and IDA, and this may be of therapeutic value. Further standardization and investigation is probably required before hepcidin levels come into routine and widespread clinical use however (Kroot *et al*, 2009).

Whether the anaemia observed in some elderly patients has the same pathogenesis as ACD has been a subject of considerable debate. In a large study of patients aged 65 years and over, the relationships between urinary hepcidin, iron status, anaemia and inflammatory markers were investigated (Ferrucci *et al*, 2010): surprisingly, urinary hepcidin levels were closely associated with markers of iron status but not with inflammatory markers, raising the possibility that hepcidin-independent pathways may contribute to hypoferraemia and anaemia in ACD, or that hepcidin levels may only be elevated in settings of overt inflammation.

### *Growth differentiation factor 15*

Growth differentiation factor 15 (GDF15) is an erythropoiesis-derived hormone that is markedly increased in β-thalassaemia and congenital dyserythropoietic anaemia, and inhibits hepcidin expression, contributing to the iron overload seen in these anaemias. Levels of GDF15 have been studied in patients with ACD, ACD/IDA and IDA (Theurl *et al*, 2010). Subjects with both ACD and ACD/IDA showed significantly higher levels of GDF15 than patients with IDA, and GDF15 concentrations correlated with interleukin-1$\beta$, suggesting that inflammation induces GDF15 expression, although the pathophysiological relevance of this is unclear.

There are currently no uniform peripheral blood laboratory criteria for the reliable diagnosis of ACD and it is therefore necessary to assess several laboratory parameters in making the diagnosis: a simplified guide to the expected patterns of various relevant investigations is therefore shown in Table II together with a diagnostic algorithm in Fig. 2.

## Management of ACD

The anaemia observed in ACD is frequently mild, and correction may not always be necessary. There are however

**Table II.** Use of laboratory investigations in the differential diagnosis of ACD.

| | ID without anaemia | IDA | ACD | ACD/IDA |
|---|---|---|---|---|
| Hb level | Normal | Low | Low | Low |
| Inflammatory markers | Negative | Negative | Raised | Raised |
| Ferritin | Low | Low | Normal/ increased | Normal |
| Transferrin saturation | Low | Low | Low | Low |
| sTFR/log ferritin ratio | Raised | Raised | Low | Raised |
| Serum hepcidin | Low | Low | Raised | Normal |
| GDF15 | Normal | Normal | Raised | Raised |

ID, iron deficiency; IDA, iron deficiency anaemia; ACD, anaemia of chronic disease; Hb, haemoglobin; sTFR, serum transferrin receptor; GDF15, growth differentiation factor 15.

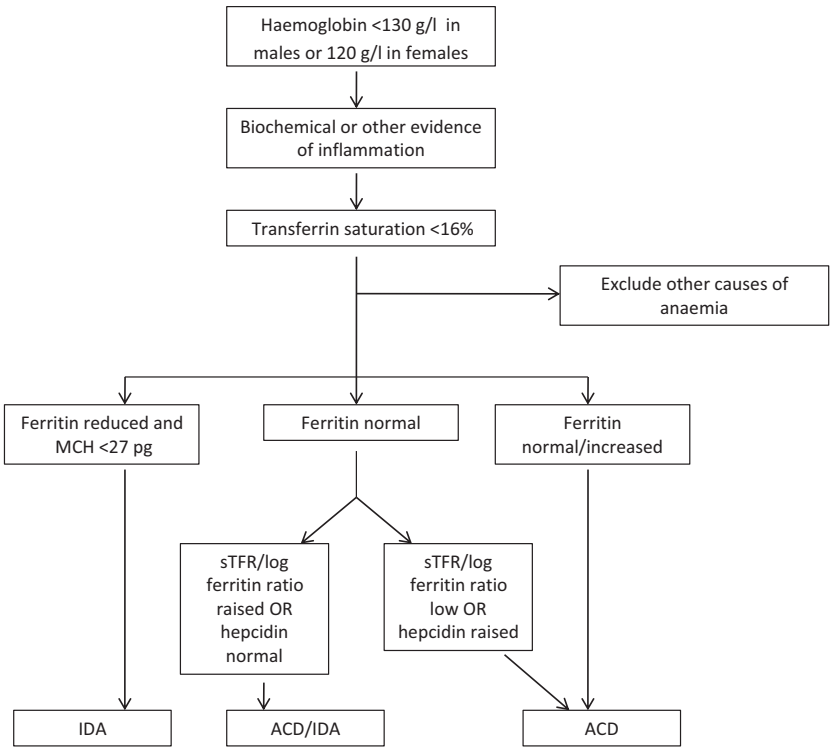


**Fig 2.** Possible algorithm for the differential diagnosis of IDA, ACD and ACD/IDA (modified from Weiss & Goodnough, 2005, with permission from the Massachusetts Medical Society© 2005). IDA, iron deficiency anaemia; ACD, anaemia of chronic disease; MCH, mean cell haemoglobin; sTFR, serum transferrin receptor.

several reasons for attempting to correct the anaemia present. Firstly, anaemia may be deleterious in itself, with effects on the cardiovascular system needed to maintain tissue oxygen supply. Secondly, anaemia may be associated with a poorer prognosis in many chronic disease states (Caro *et al*, 2001; Nissenson *et al*, 2003), although whether anaemia plays a causative role in determining prognosis is open to debate. Thirdly, treatment may improve the quality of life for patients living with chronic conditions (Moreno *et al*, 2000; Littlewood *et al*, 2001).

Treatment of the underlying inflammatory or malignant process associated with ACD will often result in improvement in the degree of anaemia, examples being the use of corticosteroids in polymyalgia rheumatica, the use of TNF-α inhibitors in rheumatoid arthritis or inflammatory bowel disease (IBD) (Moreland *et al*, 1997; Doyle *et al*, 2009; Bergamaschi *et al*, 2010), and the use of antiretroviral drugs in human immunodeficiency virus (HIV) infection (Semba *et al*, 2001). Indeed the severity of the anaemia will frequently mirror the activity of the chronic condition causing it, for example in rheumatoid arthritis (Vreugdenhil *et al*, 1990b). However, treatment of the underlying condition may not always be possible, for example in patients with incurable cancers or chronic renal or cardiac failure and alternative strategies may be necessary. Correction of as many contributory factors as possible is also desirable, for example correction of nutritional deficiencies (Vreugdenhil *et al*, 1990a).

### *Blood transfusion*

Blood transfusion is widely available in the developed world and is a simple means of treating patients with moderate to severe anaemia, but blood remains a precious and expensive resource, and transfusion therapy carries long-term risks of viral transmission, iron overload and alloimmunization. Transfusion should therefore be reserved for patients with severe or life-threatening anaemia in the context of ACD, and is not an appropriate treatment for patients with this form of chronic anaemia (Cavill *et al*, 2006).

### *Erythropoiesis-stimulating agents*

The rationale for the use of erythropoiesis-stimulating agents (ESA) in ACD is based on the blunted EPO response seen in ACD, with lower serum levels of EPO detected than would be expected for the observed degree of anaemia, together with the reduced sensitivity of erythroid progenitors to endogenous EPO seen in ACD. In addition, there is limited data to suggest that administration of EPO may reverse cytokine-mediated inhibition of erythropoiesis (Means & Krantz, 1991). Recombinant human EPO (rHuEPO) and its derivatives are widely used in patients with chronic renal failure, patients with cancer undergoing chemotherapy and patients infected with HIV on myelosuppressive anti-retroviral medication. Several different rHuEPOs are currently available or in development: epoetin-α

(Procrit®; Ortho Biotech, Bridgewater, NJ, USA; Epogen®; Amgen, Thousand Oaks, CA, USA; Eprex®; Janssen-Cilag, Cologno Monzese, Milan, Italy), epoetin-β (NeoRecormon®, F.Hoffmann-La Roche, Basel, Switzerland) epoetin-δ, biosimilar epoetins (Retacrit®; Hospira, Alemere, the Netherlands; Binocrit®; Sandoz Limited, Frimley, UK; Eporatio®; Ratiopharm, Bristol, UK), darbepoietin-α (Aranesp®; Amgen), and continuous erythropoietin receptor activator (CERA) (Mircera®; F.Hoffmann-La Roche). In addition, a PEGylated synthetic dimeric peptide capable of binding to and stimulating the EPO receptor, Hematide® (Affymax, Palo Alto, CA, USA) is undergoing clinical trials.

Much of the literature relating to the use of ESAs in ACD comes from renal medicine, but there is also evidence that these agents have useful activity in other forms of ACD, for example that seen in rheumatoid arthritis (Pincus *et al*, 1990; Peeters *et al*, 1996), IBD (Schreiber *et al*, 1996), HIV infection (Henry *et al*, 1992) and cancer (Ludwig *et al*, 1995). Only relatively small studies of EPO usage have been performed in patients with ACD secondary to inflammatory conditions, for example the study by Pincus *et al* (1990) in which four of 13 patients treated with rHuEPO at doses ranging from 50–150 iu/kg thrice weekly showed haematological responses, whereas none of four patients in the placebo arm responded. In another study (Schreiber *et al*, 1996), 34 patients with IBD refractory to iron therapy were randomly assigned to receive oral iron plus EPO or oral iron and placebo: after 12 weeks, haemoglobin levels had increased by more than 10·0 g/l in 82% of the patients in the erythropoietin group, as compared with 24% of those in the placebo group ($P = 0{\cdot}002$). However the improvement in treatment of inflammatory conditions, such as rheumatoid arthritis or IBD, with anti-inflammatory and disease modifying agents, such as TNF-α inhibitors, with associated improvements in haemoglobin levels, means that there is only a limited place for ESAs in their treatment.

There are many more studies of the use of EPO in patients with both solid tumour and haematological malignancies (Littlewood *et al*, 2001; Henke *et al*, 2003; Witzig *et al*, 2005) with response rates of 40–80% being seen. Many of these studies describe patients receiving anti-cancer treatment, so the anaemia observed may be partly due to the myelosuppressive effects of chemotherapy or radiotherapy, rather than to the inflammatory effects of malignancy alone. However, early studies indicate that, although relative EPO deficiency contributes to the anaemia of cancer in patients who are untreated, this effect is increased by the effects of chemotherapy (Miller *et al*, 1990). Smith *et al* (2003) performed a dose- and schedule determining study in 188 patients with cancer not currently receiving chemotherapy, showing responses in the majority of patients. A recent large systematic review of 46 randomized controlled trials of ESA therapy in patients with cancer concluded that patients receiving EPO had a mean 16·3 g/l higher haemoglobin level than controls, were 18% less likely to require blood transfusions, and had improved health-related quality of life, but survival benefits could not be established (Wilson *et al*, 2007).

Responses may be reduced in ACD patients with more marked inflammation (Nordström *et al*, 1997) or where there is associated iron deficiency, especially in patients with IBD (Gasche *et al*, 2004), highlighting both the importance of aiming treatment at the underlying condition and of ensuring replenishment of iron stores in patients who are iron deficient. As discussed above, it may not always be easy to determine whether patients have ACD alone or ACD/IDA, and evidence is accumulating that iron supplementation may be desirable in many patients treated with ESAs to ensure optimal response (see below).

Predicting which patients with ACD will respond to exogenous EPO would be useful, but although various algorithms incorporating baseline endogenous EPO level, early response indicators and other factors, none of these will reliably predict response, at least in the setting of cancer-related anaemia (Littlewood *et al*, 2003).

There has recently been mounting concern at possible detrimental effects of EPO administration in ACD, both in terms of cardiovascular risk and thrombosis, and relating to possible risks of tumour recurrence in patients with ACD related to malignancy. The CHOIR (Correction of Haemoglobin and Outcomes in Renal Insufficiency) study showed that trying to achieve a target Hb level of 135 g/l (compared with 113 g/l) increased the risk of cardiovascular events and did not improve quality of life (Singh *et al*, 2006) and the TREAT (Trial to Reduce Cardiovascular Events With Aranesp Therapy) study by Pfeffer *et al* (2009), demonstrated that patients with diabetes and chronic kidney disease were at greater risk of stroke following ESA administration and no clear benefit was observed. A randomized study of EPO in patients with non-small cell lung cancer (NSCLC) who were not receiving chemotherapy was terminated prematurely when a higher mortality rate was observed in the group receiving EPO (Wright *et al*, 2007).

These, and other, studies, together with suggestions that some tumour cells might express EPO receptors, raising the possibility that EPO might modulate tumour growth via cytoprotective effects, led the Food and Drug Administration (FDA) in the United States to recommend that (i) prescribers should use the lowest dose of ESAs that would gradually increase Hb concentration to a level that would avoid the need for transfusion and (ii) treatment with ESAs might increase the risk of serious cardiovascular events and death when administered to produce Hb levels >120 g/l (Jenkins, 2007). In addition, the FDA recommends that (iii) ESAs should not be used in specific tumour types (breast, head and neck, NSCLC), nor be administered to patients with active malignancy *not* receiving chemo- or radiotherapy. Similar conclusions are reached in the updated guidelines published recently by the American Society for Hematology (ASH) and the American Society for Clinical Oncology (ASCO) (Rizzo *et al*, 2010). However, a recent large meta-analysis of over 15 000 patients in 60 studies of ESAs in patients with cancer has shown no evidence that ESAs reduce survival or increase tumour

progression in patients with cancer, although some increase in venous thrombembolism was observed (Glaspy *et al*, 2010). In addition, two recent studies have cast doubt on the idea that EPO receptors may be expressed at significant and clinically relevant levels on non-haematopoietic cells, including tumour cell lines (Sinclair *et al*, 2010; Swift *et al*, 2010), and it is clear that further, well-designed, clinical trials are necessary to define the role of ESAs in the anaemia of malignancy. In the meantime, blood transfusion remains an option for treatment of anaemia in patients with contraindicated cancers or those at high risk of venous thromboembolism.

### *Iron therapy*

The recognition of the role of functional iron deficiency in the pathogenesis of ACD, together with the development of new formulations of parenteral iron, have led to a re-evaluation of iron supplementation in the management of this anaemia. As already discussed, IDA frequently co-exists with ACD, and it is clearly important that true deficiency of iron is corrected. However, even in patients with 'pure' ACD, iron supplementation may theoretically be beneficial (Goodnough *et al*, 2010). Iron deficiency may also develop during the treatment of ACD with ESAs and limit the haematological response to these agents (Kaltwasser *et al*, 2001; Cavill *et al*, 2006).

Oral iron supplements are often poorly tolerated, and patients frequently exhibit poor compliance: in addition, patients with ACD will usually have raised hepcidin levels, which would be expected to inhibit intestinal iron absorption. However, oral iron is cheap, widely available, and easy to administer, and given the difficulties in ruling out concomitant IDA in many patients with ACD, a therapeutic trial of oral iron will be undertaken by many clinicians treating ACD. It must however be recognized that failure to respond to oral iron rules out neither true, nor functional iron deficiency.

There is little literature on the use of intravenous iron supplementation alone in the treatment of ACD. Cazzola *et al* (1996) reported on the beneficial effects of intravenous (IV) iron in 20 consecutive patients with juvenile chronic arthritis, although it is likely that a significant proportion of these patients also had true iron deficiency. Studies in patients with gynaecological cancer (Kim *et al*, 2007; Dangsuwan & Manchana, 2010) also showed a benefit in terms of reduced transfusion requirements for those receiving intravenous iron supplementation. However baseline iron status was not reported in either of these papers, and clearly larger studies are needed.

Much of the literature concerning intravenous iron supplementation has come from the field of renal medicine, where the superiority of parenteral over oral iron supplementation is now well established, and not only improves the responses to ESAs but can also lead to reduced doses of ESAs being used (Locatelli *et al*, 2009). The DRIVE (Dialysis Patients' Response to IV iron and with Elevated Ferritin) trial (Coyne *et al*, 2007) randomized selected haemodialysis patients with elevated ferritin and reduced transferrin saturation to receive or not receive intravenous ferric gluconate together with EPO. The patients who received IV iron showed more rapid and better responses in Hb level than the controls, and similar responses were seen in patients with transferrin saturations above and below 19%, leading the authors to conclude that functional iron deficiency was a significant contributor to anaemia in this setting, and that this could be overcome by intravenous iron supplementation.

There is now evidence that intravenous iron can enhance the effects of ESAs in patients with other forms of ACD, particularly cancer-related anaemia (reviewed by Littlewood & Alikhan, 2008). Auerbach *et al* (2004) randomized 155 patients being treated with ESAs for chemotherapy-related anaemia to no iron, oral iron or intravenous iron: there were significant improvements in haematological responses in patients receiving intravenous iron compared with those receiving either no iron or oral iron. These observations have been confirmed in several subsequent studies (Hedenus *et al*, 2007; Henry *et al*, 2007; Bastit *et al*, 2008; Pedrazzoli *et al*, 2008). Criteria for exclusion of co-existent IDA varied between these trials, and it is possible that significant numbers of patients included were in fact iron deficient, but the study by Hedenus *et al* (2007) is of particular interest as it enrolled only patients with lymphoproliferative malignancies not receiving chemotherapy, and all patients had detectable bone marrow iron stores.

In contrast, a recent study by Steensma *et al* (2011) randomized patients with chemotherapy-associated anaemia to no iron, oral iron or intravenous iron plus darbepoietin: all had serum ferritin >20 $\mu$g/l and transferrin saturations <60%. There was no difference in erythropoietic response between the three groups. The mean pre-treatment ferritin levels in this study were higher than in the other studies, suggesting this population was less likely to have co-existent IDA, and the doses and scheduling of iron infusions were lower. Both these observations may partly explain the different results observed, but it is clear that further prospective studies, with better characterization of baseline iron stores are needed to define the role of intravenous iron supplementation in this setting. The ASH/ASCO guidelines (Rizzo *et al*, 2010) recommend periodic monitoring of iron status in patients receiving treatment with ESAs but fall short of recommending intravenous supplementation to augment responses.

It is not yet known how intravenous iron might overcome the reticuloendothelial blockade on iron utilization thought to be fundamental to the pathogenesis of ACD, but it is possible that the infused iron may become bound directly to transferrin rather than being taken up by macrophages, and is thus available to the erythron. There are however no *in vitro* data to support this hypothesis.

Safety issues also need to be considered when using intravenous iron, particularly as older preparations were associated with significant adverse events, including anaphylaxis (Auerbach & Ballard, 2010). Recent pharmacological

developments have led to the release of several new iron formulations including low molecular weight iron dextran (Cosmofer®; Pharmacosmos, Holbaek, Denmark), iron sucrose (Venofer®; Vifor Pharma, Glattbrugg, Switzerland), ferric carboxymaltose (Ferinject®; Syner-Med Ltd, Purley, UK) and sodium ferric gluconate (Ferrlecit®; Watson Laboratories, Morristown, NJ, USA). In the trials above, no excess of adverse effects was observed with these newer intravenous iron preparations. One hypothesis for the hypoferraemia seen in ACD is that low iron levels might inhibit bacterial growth, as iron is essential for the growth and survival of intracellular bacteria, but there is no evidence to date that supplemental iron increases the risk of infections. However the long-term effects of intravenous iron administration on other parameters, for example tumour growth and cardiovascular disease, have not been studied.

## Possible future directions

### *Direct suppression/blockade of hepcidin activity*

*Anti-hepcidin antibodies.* Experiments *in vitro* and *in vivo* in a murine model of ACD suggest that suppression or inhibition of hepcidin expression may be a possible means of modulating ACD (Sasu *et al*, 2010): overexpression of human hepcidin in mice produces a picture of anaemia similar to that seen in ACD, with resistance to exogenous EPO therapy, and mice rendered anaemic by heat-killed *Brucella abortus* were effectively treated by hepcidin mRNA suppression. Treatment of mice overexpressing human hepcidin with anti-hepcidin antibodies did not by itself lead to resolution of treated animals, but did restore sensitivity to treatment with EPO. Such a treatment might therefore obviate the need for intravenous iron therapy to supplement EPO in patients with ACD.

### *Indirect suppression of hepcidin*

Dorsomorphin is a small molecule inhibitor of BMP signalling that was identified during screening of compounds that dorsalize zebrafish embryos. *In vitro* experiments showed inhibition by dorsomorphin of BMP, IL-6 and haemojuvelin-stimulated expression of hepcidin, and *in vivo* inhibition of iron-stimulated expression of hepcidin mRNA in zebrafish, and induction of hyperferraemia in iron-replete mice (Yu *et al*, 2008), suggesting a possible role in reducing elevated hepcidin levels in ACD.

Similarly, in a murine model of inflammatory bowel disease, inhibition of BMP by HJV.Fc, a recombinant protein that prevents binding of BMPs to their receptor, LDN-193189, a small molecule inhibitor of BMP signal transduction, and an anti-BMP-6 antibody, inhibited hepcidin expression and increased serum iron levels (Wang *et al*, 2011).

Finally heparin is known to bind BMPs, and can variably modulate their signalling effects: exogenous heparin has recently been shown to downregulate hepcidin expression by the hepatoma cell line, HepG2 (Poli *et al*, 2011) in a dose-dependent manner, and at pharmacological concentrations. Treatment of mice with heparin inhibited liver hepcidin mRNA expression and SMAD phosphorylation, reduced spleen iron concentration, and increased serum iron, and administration of heparin to five patients with deep venous thrombosis also produced reductions in hepcidin levels. The authors postulated that the effects of heparin in their study were mediated by sequestration of BMP proteins, with formation of complexes that are unable to stimulate SMAD signalling and hepcidin expression, and further studies of its potential in improving haemoglobin levels in patients with ACD seem warranted for this widely used agent.

*Anti-IL-6 receptor antibodies.* Castleman disease is a rare lymphoproliferative disorder characterized by hyperplastic lymph nodes showing follicular hyperplasia and capillary proliferation associated with endothelial hyperplasia. Dysregulated production of IL-6 has been shown to be responsible for some of the systemic manifestations of the multicentric form of the disease (MCD). Five out of six patients with MCD receiving longterm treatment with an anti-IL-6 receptor antibody, tocilizumab, showed rapid reductions in serum hepcidin levels, and a more gradual, but progressive, improvement in haematological parameters, including anaemia, was observed in nine patients (Song *et al*, 2010). Anti-IL-6 receptor blockade may represent a future targeted therapy for ACD.

*Vitamin D.* A recent study (Perlstein *et al*, 2011) has shown an association between vitamin D deficiency and ACD in the elderly: the Third National Health and Nutrition Examination Study (NHANES III) examined health and nutritional status of non-institutionalized subjects over 60 years of age in the United States of America, and haemoglobin and vitamin D levels were obtained in 5100 and 4575 subjects respectively. A significant correlation between haemoglobin and vitamin D levels was found. Further analysis of a subset of 2610 patients, who had more detailed haematological data available, showed that this association was particularly strong for patients with ACD. Individuals with ACD were twice as likely to have vitamin D deficiency as non-anaemic subjects. It remains to be seen if there is an aetological link between vitamin D deficiency and ACD, and whether vitamin D replenishment will have any therapeutic role to play.

*Pentoxifylline.* Pentoxifylline is a drug with anti-inflammatory properties, and can suppress production of TNF-$\alpha$ and IFN-$\gamma$. Two studies have suggested a beneficial effect for this agent in chronic renal failure patients with anaemia resistant to EPO (Cooper *et al*, 2004; Ferrari *et al*, 2010): parallel reductions in pro-inflammatory cytokine levels suggested that the mechanism for this effect was via the anti-inflammatory effects of this agent, and future studies in ACD may be worthwhile.

## Conclusions

The anaemia in ACD contributes hugely to the morbidity experienced by millions of patients worldwide suffering from a large variety of inflammatory, infective and malignant conditions. Recent years have seen a marked expansion in our understanding of the pathogenesis of ACD, particularly in the key role played by hepcidin in mediating the functional iron deficiency that is the hallmark of this very common form of anaemia. This is likely to produce future improved diagnostic tests for ACD, which frequently co-exists with, and may be difficult to distinguish from, other forms of anaemia. It is hoped that this may also lead to the development of more specific treatments targetting the molecular pathways involved in the pathogenesis of ACD.

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