

**ABSTRACT**

Anaemia of chronic disease (ACD) or anaemia of chronic inflammation may be secondary to infections, auto immune disorders or malignancies. It is characterized by an immune activation with an increase in inflammatory cytokines and resultant increase in hepcidin levels. In addition, inappropriate erythropoietin levels or hyporesponsiveness to erythropoietin and reduced red blood cell survival contribute to the anaemia. Hepcidin being the central regulator of Iron Metabolism plays a key role in the Patho physiology of ACD. Hepcidin binds to the Iron export protein, ferroportin, present on macrophages, hepatocytes and enterocytes, causing degradation of the later. This leads to iron trapping within the macrophages and hepatocytes, resulting in functional Iron deficiency. Production of hepcidin in turn regulated by iron stores, inflammation and erythropoiesis via the BMP-SMAD and JAK-STAT signalling pathways. Treatment of anaemia should primarily be directed at the underlying disease, and conventional therapy such as red blood cell transfusions, iron, erythropoietin and novel agents targeting the hepcidin ferroportin axis and signalling pathways (BMP6-HJV-SMAD and IL-6-JAK-STAT) involved in hepcidin production also may be considered.

**INTRODUCTION**

Anaemia of chronic disease (ACD) or Anaemia of chronic inflammation is the term used to describe the hypoproliferative anaemia seen in response to chronic infection, chronic immune activation and malignancy. It is the second most prevalent form of anaemia after iron deficiency anaemia (IDA).<sup>1</sup> The anaemia is typically normochromic, normocytic and mild in degree. However other observations have shown that ACD can be seen in a variety of other Conditions including severe trauma, diabetes mellitus and anaemia of older adults.

All these condition produce massive elevation of interleukin-6 (IL-6), which stimulates Hepcidin production & release from the liver which in turn reduces the Iron Carrier protein, ferroportin,<sup>2</sup> so that access of Iron to the circulation is reduced as well as iron absorption in the small intestine, iron transport across the placenta and release from the macrophages are also reduced. Also there is impaired production of erythropoietin (EPO), blunted marrow erythroid response to EPO, Iron restricted erythropoiesis and a diminished pool of EPO- responsive cells.<sup>3</sup>

**EPIDEMIOLOGY**

ACD is considered the second most common cause of anaemia world wide however detailed statistics on its prevalence are not available. Often the anaemia in individuals with inflammatory diseases is complex and multifactorial<sup>4</sup> and it may be challenging to separate out the component due to ACD. This is especially true in patients with diabetes. Examples of the prevalence of ACD in various inflammatory states include the following :

- Anaemia is observed in 33 to 60 % of patients with rheumatoid arthritis.<sup>5</sup>
- ACD accounts for about 1/3<sup>rd</sup> of the cases of anaemia of the elderly because of concomitant inflammatory conditions or chronic kidney diseases.
- Cancer elated anaemia occurs in more than 30% of the cases at diagnosis,<sup>6</sup> the rate reached 63% in an observational study on 888 consecutive cancer patients. however cancer related anaemia is multifactorial and includes types of anaemia other than ACD (eg IDA). Anaemia is even more common in haematologic malignancies like lymphoma and multiple myeloma.

**ACUTE VARIANT OF ACD**

Acute event related anaemia, such as that occruing after surgery, major trauma, myocardial infarction or sepsis, a condition called the Anaemia of Critical illness, shows many of the features of ACD (i.e. low serum Iron, high ferritin, blunted response to EPO), presumably secondary to tissue damage and acute inflammatory changes.<sup>7</sup> It appears to be an acute variant of ACD and is also characterized by shortened RBC survival.<sup>8,9</sup>

**Anaemia with Chronic Obstructive Pulmonary Disease**

A subset of patients with chronic obstructive pulmonary disease, estimated at approximately 50%, have laboratory findings consistent with ACD (eg, anaemia, elevated levels of CRP, IL-6, IFN  $\gamma$  and low serum erythropoietin) suggesting the presence of back ground infection or inflammation.

**Anaemia with chronic kidney disease**

Anaemia with chronic kidney disease shares some of the characteristics of ACD, although the decrease in the production of erythropoietin, mediated by renal insufficiency and the anti proliferative effects of accumulating uremic toxins, contribute importantly.<sup>10</sup>

In addition, in patients with ESRD, chronic immune

**Table 1: 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

activation can arise from contact activation of immune cells, by dialysis membranes, from frequent episodes of infection or from both factors, and such patients present with changes in the homeostasis of body iron that is typical of anaemia of chronic disease.

### Diseases associated with ACD

Conditions associated with ACD are listed in Table 1. These diseases all share features of acute or chronic immune activation.

### Pathophysiological features

Anemia of chronic disease is immune driven; cytokines and cells of the reticuloendothelial system induce changes in iron homeostasis, the proliferation of erythroid progenitor cells, the production of erythropoietin, and the shortened life span of red cells, all of which contribute to the pathogenesis of anemia.<sup>11</sup> Erythropoiesis can be affected by disease underlying anemia of chronic disease through the infiltration of tumor cells into bone marrow or of microorganisms, as seen in human immunodeficiency virus (HIV) infection, hepatitis C, and malaria.

Moreover, tumor cells can produce proinflammatory cytokines and free radicals that damage erythroid progenitor cells. Bleeding episodes, vitamin deficiencies (e.g., cobalamin, folic acid and vitD), hypersplenism, autoimmune hemolysis, renal dysfunction, and radio- and chemotherapeutic interventions themselves can also aggravate anemia.

There are sufficient data and findings that suggest that hepcidin may be central to the anaemia of chronic disease. A recently identified gene 'HEMOJUVELIN' may act in

concert with hepcidin in inducing the changes found in ACD.<sup>12</sup>

### Causes and Pathogenesis

A. Altered iron homeostasis with Inflammation and Hepcidin including role of cytokines

It has been suggested that the underlying inflammatory medical condition causes the release of cytokines such as the interleukins (IL-1 & IL-6) and tumor necrosis factor-alpha by activated monocytes, these cytokines unleash a cascade which include the secretion of interferon (IFN-B and IFN- $\gamma$ ) by T-lymphocytes. As an example, IFN gamma h when given to experimental animals, can produce picture of ACD.

The decreased bone marrow responsiveness to erythropoietin is mediated by inflammatory cytokines.

Especially IL-1 beta and TNF- $\alpha$  which may induce apoptosis of red cell precursors as well as down regulation of erythropoietin receptors on progenitor cells. Cytokines may also decrease erythropoietin expression by renal cells.<sup>13</sup> In vitro treatment of cultured cells with proinflammatory cytokines can also alter ferritin and transferrin receptor expression and Iron-responsive protein activity in macrophages.

Studies suggest that IL-6 is required for the induction of hepcidin and hypoferraemia during inflammation in both animals and humans, although hepcidin can also be upregulated by the cytokine IL -1, while the molecular mechanisms responsible for this activation are only partially understood, IL-6 appears to be involved in the regulation of hepcidin levels through the JAK-stat 3 signalling pathway.

- B. Reduced EPO production: Under normal physiologic 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.
- C. Reduced Erythroid Responsiveness (Impaired proliferation of EP cells): In ACD, the proliferation and differentiation of Erythroid Progenitor (EP) cells is reduced. Hepcidin itself has an inhibitory effect on erythropoiesis in vitro at low EPO concentrations.

In ACD both groups of erythroid precursors erythroid burst forming units and erythroid colony forming units are impaired and are linked to inhibitory effects of IFN-alpha, beta and gamma, TNF-alpha and interleukin -1 which influence the growth of erythroid burst forming units and erythroid colony forming units. IFN-gamma appears to be most potent inhibitor.<sup>14</sup>

The underlying mechanisms may involve cytokine

**Table 2: Serum levels that differentiates Anemia of Chronic disease from iron deficiency anemia**

Variable	Anemia of Chronic Disease	Iron-Deficiency Anemia	Both Conditions <sup>†</sup>
Iron	Reduced	Reduced	Reduced
Transferrin	Reduced to normal	Increased	Reduced
Transferrin saturation	Reduced	Reduced	Reduced
Ferritin	Normal to increased	Reduced	Reduced to normal
Soluble transferrin receptor	Normal	Increased	Normal to increased
Ratio of soluble transferrin receptor to log ferritin	Low (<1)	High (>2)	High (>2)
Cytokine levels	Increased	Normal	Increased

\*Relative changes are given in relation to the respective normal values. †Patients with both conditions include those with anemia of chronic disease and true iron deficiency.

mediated induction of apoptosis. Moreover, cytokines exert direct toxic effects on progenitor cells by inducing the formation of labile free radicals such as nitric oxide or superoxide anion by neighbouring macrophage like cells.

### REDUCED RED CELL SURVIVAL

Red cell survival is modestly shortened in patients of Rheumatoid Arthritis and other conditions, 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.

### Diagnostic issues in ACD

The widespread settings in which ACD may be seen can make diagnosis difficult. Typically the anaemia is mild to moderate, Hb Conc. 10 to 11 gm/d l normochromic and normocytic (although anaemia may become microcytic as disease progresses) and the reticulocyte count is low, the absolute reticulocyte count is <25000/micro L & reticulocyte Hb content is <28 pg/dl. The anaemia may be accompanied by an elevation of cytokines (IL-6) as well as acute phase reactants (eg fibrinogen, ESR, CRP, ferritin, haptoglobin factor, viii) reflecting the hypoproliferative nature of the anaemia.

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.

If the distinction between ACD and ACD/IDA cannot be made by laboratory tests alone, one may monitor the response to a short trial (4-6 wks) of oral iron supplementation.

Measurement of erythropoietin levels is useful only for anemic patients with hemoglobin levels of less than 10 g per deciliter, since erythropoietin levels at higher hemoglobin concentrations remains well in the normal range.

The measurement of Serum transferrin receptor (sTFR), the truncated fragment of the membrane receptor, has been suggested as a possible tool for differentiating between ACD and IDA. The ratio of sTFR to the log of the serum ferritin (sTFR/ferritin ratio) has been proposed to be a useful tool in the diagnosis of ACD, and particularly in differentiating ACD from IDA. This ratio is effective in making this distinction since STfr is increased in IDA and normal in ACD while ferritin or log ferritin is decreased in IDA and normal to increased in ACD. Specifically a sTfr/ log ferritin ratio < 1 suggests ACD while a ratio >2 suggests IDA. Those with combination or ACD & IDA will also have a Tfr-ferritin index >2.<sup>13</sup>

Hepcidin Assays-Assays to measure serum Hepcidin are not yet routinely available for clinical use. In one study measurement of Hepcidin-25 level by mass spectrometry was proposed as a potential tool for differentiating ACD from IDA.<sup>14</sup> The use of a Hepcidin-25 cut off  $\leq 4$ nmol/L allowed the differentiation of IDA from ACD.

Bone marrow picture-Examination of bone marrow for its content and distribution of Iron is instructive, although this examination is not routinely performed in all patients with suspected ACD. In the most classical presentation of ACD, bone marrow macrophages contain normal or increased amounts of storage Iron, reflecting Reduced export of iron from macrophages due to the action of hepcidin. In addition, erythroid precursors show decreased or absent Staining for Iron (ie, decreased no. of sideroblasts) reflecting reduced availability of Iron for Redcell production.<sup>15</sup>

### Differential Diagnosis by Bone marrow study

While bone marrow examination is not required for most patients in whom ACD is suspected, in difficult cases the diagnosis can often be established by bone marrow examination. Findings in the most common disorders include:

- ACD - Bone marrow macrophages contain normal to increased Iron, while erythroid precursors show

**Table 3: Therapeutic Options for the Treatment of Patients with Anemia of Chronic Disease**

Treatment	Anemia of Chronic Disease	Anemia of Chronic Disease with True Iron Deficiency
Treatment of underlying disease	Yes	Yes
Transfusions*	Yes	Yes
Iron supplementation	No	Yes <sup>†</sup>
Erythropoietic agents	Yes <sup>‡</sup>	Yes, in patients who do not have a response to iron therapy

\*This treatment is for the short-term correction of severe or life-threatening anemia. Potentially adverse immunomodulatory effects of blood transfusions are controversial. <sup>†</sup>Although iron therapy is indicated for the correction of anemia of chronic disease in association with absolute iron deficiency, no data from prospective studies are available on the effects of iron therapy on the course of underlying chronic disease. <sup>‡</sup>Overcorrection of anemia (hemoglobin >12 g per deciliter) may be potentially harmful to patients: the clinical significance of erythropoietin-receptor expression on certain tumor cells needs to be investigated.

decreased to absent amounts of Iron (ie decreased to absent sideroblasts).

- IDA-stainable Iron is absent from both macrophages and erythroid precursors.

### MYELODYPLASTIC SYNDROME

Single or multilineage dysplastic changes with or without increased number of sideroblasts, including ring forms, are commonly seen in myelodysplasia.

### SIDEROBLASTIC ANAEMIA

The diagnostic hallmark of the sideroblastic anaemias is the presence of ring sideroblasts on bone marrow examination. The amount of Iron in bone marrow macrophages is strikingly increased due to the presence of ineffective erythropoiesis. Single or multilineage dysplastic changes are not seen.

### Management of ACD

The anaemia observed in ACD is frequently mild, and correction may not always be necessary. Treatment of the underlying inflammatory or malignant process associated with ACD will often result in improvement in the degree of anaemia.

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. Thirdly, treatment may improve the quality of life for patients living with chronic conditions.

### Treatment options

In cases in which treating the underlying disease is not feasible, alternative strategies are necessary (Table 3).

### Blood transfusion

Blood transfusion 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.

### 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,<sup>16</sup> 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.

Measurement of serum EPO concentration may be helpful in patients with ACD who have symptomatic anaemia. and/or who have not responded to treatment of their underlying disorder and continue to have symptomatic anaemia requiring treatment.

Recombinant human EPO (rHuEPO) and its derivatives are widely used in patients with chronic renal failure, patients with cancer undergoing chemotherapy and in patients infected with HIV on myelosuppressive anti-retroviral medication. Several different rHuEPOs are currently available or in development: Haematology 2011, 154, 289–300]. epoetin- $\alpha$ , epoetin- $\beta$ , epoetin- $\delta$ , biosimilar epoetins, darbepoetin- $\alpha$ .

The percentage of patients with anemia of chronic disease who respond to therapy with erythropoietic agents is 25 percent in myelodysplastic syndromes, 80 percent in multiple myeloma, and up to 95 percent in rheumatoid arthritis and chronic kidney disease. The therapeutic effect involves counteracting the antiproliferative effects of cytokines, along with the stimulation of iron uptake and heme biosynthesis in erythroid progenitor cells.

Dosage of EPO- Although one of the hallmarks of ACD is a reduced erythropoietic response to both endogenous as well as exogenous EPO, high doses of EPO may overcome this hyporesponsiveness.<sup>17</sup>

### TWO TREATMENT OPTIONS ARE AVAILABLE

- standard dosing of EPO is a start up dose of 100 to 150 units /kg, subcutaneous three times weekly along with Supplemental iron. Responders may show a rise in the haemoglobin concentration of at least 0.5 gm/dl by two to four wks. If there is no elevation in the Hb concentration by six to eight weeks. Then the regimen can be intensified to daily therapy or 300 units /kg three times weekly. It is not worth while to continue EPO in patients who

do not have clinically meaningful response by 12 weeks.

- B. An alternative treatment schedule is to employ 30000 to 40000 units of EPO given subcutaneously once per week,<sup>18</sup> a single dose that is numerically equivalent to a dose of 140 to 190 units/kg three times per week for a 70 kg person. the dose can be increased to 60000 units if there is no response (ie Hb rise <1 gm/dl) at four weeks.

For ease of use and to minimize inconvenience to the patient, the second schedule is preferred.

Potential adverse effects of EPO therapy can be minimised by initiating treatment when the patients Hb% is <10 gm/dl and stopping treatment when Hb% reaches 12gm/dl.

Darbepoietin: Although darbepoietin has had limited use in the treatment of ACD cases in humans, it is capable of reversing anaemia due to chronic Inflammatory disease in experimental animals. A dose of darbepoietin equivalent to the above noted dose of EPO is in the range of 60 to 100 microgm/wk or 300 ugm every 3 weeks. However, if we are looking for a rapid, short term response of the anaemia, darbepoietin with its prolonged half life is less preferred.

## IRON THERAPY

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.

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. There is now evidence that intravenous iron can enhance the effects of ESAs in patients with other forms of ACD, particularly cancer-related anaemia. 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.

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.

Recent developments have led to the release of several new iron formulations including low molecular weight iron dextran, iron sucrose, ferric carboxymaltose and sodium ferric gluconate. In the trials, no excess of adverse effects was observed with these newer intravenous iron preparations.

## Novel Agents for the Future

The increased production of hepcidin in ACD serves as the rationale for development of inhibitors targeting the BMP-HJV-SMAD and IL-6-JAK-STAT pathway that are involved in hepcidin synthesis.

- Dorsomorphin, is a small molecular inhibitor of BMP receptor and LDN-193189,<sup>19</sup> a more selective BMP inhibitor has been tried.
- Other agents include anti- BMP- 6 monoclonal antibody and soluble HJV. In addition heparin has also been shown to decrease BMP-SMAD signalling<sup>20</sup> and inhibit hepcidin transcription.
- Iron chelation therapy has also been advocated to induce endogenous formation of EPO.
- Also in the development are hepcidin antagonists<sup>20</sup> (monoclonal antibodies, small interfering RNA [SiRNA], antisense oligonucleotides, hepcidin binding proteins and aptamers). Anti hepcidin monoclonal antibodies bind to hepcidin and prevent it from binding to ferroportin.<sup>21</sup>
- In addition hepcidin binding proteins, anticalines and spiegelmers are also being developed<sup>22</sup> the anti hepcidin spiegelmers NOX-H94 is in a phase II-A clinical trial for the treatment of ACD with a very promising result.
- In regards to the IL- 6- JAK-STAT pathway, monoclonal IL-6 and IL-6R<sup>23</sup> antibodies (siltuximab and tocilizumab) and JAK-2 and STAT-3 inhibitors, all have been shown to down regulate hepcidin expression.
- Vit D deficiency is associated with an increased prevalence of ACD, and vit D replacement lowers hepcidin levels.
- Since hepcidin excess blocks ferroportin by causing degradation of the latter, agents that stabilize ferroportin or inhibit the interaction with hepcidin are an attractive target which will pave the way for the development of anti-ferroportin monoclonal antibody that blocks hepcidin ferroportin interaction.

## Monitoring Therapy

Before the initiation of therapy with an erythropoietic agent, iron deficiency should be ruled out.

For monitoring the response to erythropoietic agents, hemoglobin levels should be determined after four weeks of therapy and at intervals of two to four weeks thereafter. If the hemoglobin level increases by less than 1 g per deciliter, the iron status should be re-evaluated and iron supplementation considered. If iron-restricted

446 erythropoiesis is not present, a 50 percent escalation in the dose of the erythropoietic agent is indicated. The dose of the erythropoietic agent should be adjusted once the hemoglobin concentration reaches 12 g per deciliter. If no response is achieved after eight weeks of optimal dosage in the absence of iron deficiency, a patient is considered nonresponsive to erythropoietic agents.

## CONCLUSION

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.

Future strategies may include the use of iron-chelation therapy to induce the endogenous formation of erythropoietin, hepcidin antagonists that overcome the retention of iron within the reticuloendothelial system, and hormones or cytokines that might effectively stimulate erythropoiesis under inflammatory conditions.

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