

# The pathogenesis of anemia of chronic disorders

C.U. Manzini, E. Manzini, C. Ferri

U.O.C. di Reumatologia, Azienda Ospedaliero-Universitaria, Modena, Italy

## SUMMARY

Anemia of chronic disorders is a typical condition of infective, immunological and neoplastic diseases. Hepcidin and proinflammatory cytokines play a leading role in its pathogenesis. Hepcidin is a hormone produced by the liver that controls iron metabolism. It ensures that iron is retained by enterocytes (where the metal is absorbed) and by macrophages (that store the iron that results from the physiological breakdown of erythrocytes). Cytokines play a role in hepcidin synthesis, and in the proliferation and the maturation of the erythroid components within bone marrow. This paper discusses the pathogenetic mechanisms of anemia in chronic disorders.

**Key words:** Anemia of chronic disorders, hepcidin, proinflammatory cytokines.

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## ■ INTRODUCTION

Anemia of chronic diseases (ACD) has a complex etiopathogenesis that presents itself during the course of infective, immune and neoplastic pathologies. Rheumatoid arthritis (RA) is often considered to be a prototypic disease of ACD. This type of anemia is characterized by a slight or moderate normochromic-normocytic anemia with hemoglobin 9-11 g/dL, complete blood count 30-40% and by normal or slightly lower than normal reticulocyte count. Biochemical parameters show a reduction in serum iron (essential for a diagnosis of ACD), low transferrin saturation and a decrease in total iron binding capacity, in contrast to true iron deficiency in which there is an increase in total iron binding capacity. Furthermore, increased ferritin confirms that, in spite of the hypsideremia, the organic iron reserve is normal or higher than normal.

Bone marrow cellularity is, on the whole, normal with scarce or a complete lack of erythroblastic hyperplasia. Non-hemoglobin iron staining (Perls reaction) shows a sharp reduction in normal values and an increase in macrophages containing hemosiderin.

In patients with ACD, and in particular in female patients of child-bearing age or in cases with true iron deficiency (hemorrhage, reduced dietary intake or malabsorption of iron), a true hypochromic microcytic anemia is sometimes established. Anemia in rheumatoid arthritis (RA) correlates with the degree of disease activity but not disease duration. (1) The different mechanisms that determine ACD have still not been completely defined. However, these have been thought to be an alteration in iron metabolism, a slight reduction in the average erythrocyte age and in a modified proliferative response in the bone marrow erythroid compartment. (2)

### *Alteration in iron metabolism*

A radical alteration in iron metabolism is seen by the presence of hypsideremia associated to adequate or increased iron in depository organs (bone marrow, liver and spleen), but also in the inflamed synovial fluid (3).

The best supported hypothesis to explain hypsideremia is that the iron stored in the monocyte-macrophage system, resulting from the physiological destruction of aged erythrocytes, is not released in sufficient quantities to the plasma transferrin, that

Corresponding author:  
Carlo Umberto Manzini  
U.O.C. Reumatologia

Azienda Ospedaliero-Universitaria  
Via del Pozzo, 71 - 41100 Modena, Italy  
E-mail: manzini.carlo@policlinico.mo.it

has also been decreased. In fact, the inadequate supply of iron to the erythron will limit the hemoglobin-synthetic capacity of the erythroblasts.

Recent experimental research on animals and on humans has contributed enormously to our understanding of organic iron homeostasis both in normal conditions and in a variety of metabolic deficiency pathologies. To explore the alterations in iron metabolism presented in ACD it is useful to take a brief look at the principle mechanisms that regulate iron absorption and its use in normal subjects.

Metabolic iron deficiency has two main characteristics. Firstly, in physiological conditions, once iron has entered the organism it is only released in small quantities, although women lose more through their menstrual cycle. Secondly, iron homeostasis must be maintained to satisfy the organisms's physiological requirements and this maintenance is managed exclusively by absorption of iron by the intestine (4) and not through changes in excretion processes, as happens with other substances.

Iron is present in the diet in two forms:

1. inorganic (non-hemoglobin iron), to be found in all types of food but particularly in vegetables; and
2. organic (hemoglobin iron), derived from the breaking up and processing of the hemoglobin and myoglobin to be found in red meat (4).

The proximal gastrointestinal tract is the best site for optimal and elective iron absorption.

Approximately 80-90% of dietary iron is in an inorganic trivalent form. In order to be absorbed, it must be transformed into a bivalent form by an iron reductase (duodenal cytochrome b-Dcytb) (5) present on the surface of the enterocytes or through another mechanism (6). In this form, it is transferred inside the epithelial cells of the duodenum and of the proximal jejunum by a transport protein (divalent metal transporter 1-DMT1) (7). Here the metal binds to apoferritin and forms ferritin. It is in this form that most of the absorbed iron is eliminated through cleavage of the parietal cells in the intestinal lumen. Only a small

quantity of absorbed iron (1-2 mg, varying according to organic requirements) (8, 9) will be reconverted into a trivalent form by an iron-oxidase, hephaestin, in order to be used (10). It is later released to the ferroportin (a protein carrier present on the surface of the laterobasal membrane of the intestine) that takes it into circulation. Here the iron binds to plasma transferrin that carries it to where it will be used or to the depository organs where it is stored in the form of ferritin or hemosiderin. Ferroportin is also abundantly expressed on macrophages of the sterol regulatory element (SRE) of the liver, of the spleen and of the bone marrow showing that this protein also carries iron from the cells, that they recycle the iron from aged erythrocytes (11) and, at the same time, confirms the existence of a common iron release mechanism both by macrophages and by enterocytes.

However, there are different ways in which the iron present in heme is absorbed by the intestine. This iron is derived from the breakdown of hemoglobin (Hb) in that, in order to be absorbed, it must bind to a transport protein in the brush-border of the duodenum enterocytes (11) that carries it intact to the inside of the epithelial cells. Here the iron is transformed into a bivalent form by heme-oxygenase (11) and, together with the absorbed inorganic iron (non-heme Fe), enters the low weight molecular pool located in the enterocyte. Then, in the same way as for inorganic iron, iron from heme is released by the ferroportin to the plasma transferrin or eliminated through cleavage of the cells in the intestinal lumen (11). To distribute the iron to the cells, the plasma transferrin must bind to a specific receptor (TFR) found on them and, in this form, the complex is embedded in their cytoplasm where the iron is released and used. The cells of the erythroid compartment within the bone marrow are those that express the greatest number of transferrin receptors on their surface.

The soluble transferrin receptor (sTbR), a fragment derived from the proteolysis of receptor tissues, circulates in the form of a transferrin-receptor complex and is considered a sensitive indicator of organic iron

depletion. Table I shows the main characteristics that differentiate between anemia of chronic diseases and anemia of true iron deficiency.

The discovery of hepcidin has been of fundamental importance in the field of metabolic iron deficiency. This is a peptide that is synthesized in the liver and that can be found in the blood and in urine. Besides its bacterial action, it also plays an essential role in organic iron homeostasis (12-14). From research carried out on animal models and in human pathologies, it has been seen that hepcidin stops iron being released from the enterocytes, where iron is absorbed, and from the macrophages, cells in which iron derived from the physiological breakdown of erythrocytes is stored. In order to do this, hepcidin must bind to ferroportin (the only known iron transporter, present on the cell surface of enterocytes, macrophages and hepatocytes) that determines access and subsequent breakdown inside the cells themselves. In fact, it has been found that if concentrations of circulating hepcidin are low, the ferroportin that is normally expressed on the cells of the deficient deposits can carry and release to the plasma transferrin the amount of iron needed to satisfy the organisms's requirements. Instead, the opposite happens if there are large amounts of the circulating peptide since a deficiency of ferroportin, induced by the hepcidin at a cellular level, results in less iron being exported from the deposits, therefore, establishing hyposideremia (15).

Other studies have shown that the variations in circulating hepcidin concentrations observed in phlogistic and infective pathologies are mediated by proinflammatory cytokines (16-18) and in particular, IL6.

This binds to its receptor, activates STAT3 (signal transducer and activator of transcription), the main factor that regulates the peptide hepatic synthesis, increases hepcidin production and, consequently, as seen in ACD, reduces the amount of iron available for use (18, 19). Although IL6 is known to be essential to inducing hepcidin synthesis in inflammatory pathologies, it has been found that STAT3 can also be mediated by other factors (20).

This is why hepcidin also plays such an important role in immune response to infections since the increase in the amount of circulating peptide that is observed in these pathologies denies the pathogenic agents the iron that they need to survive (21). Given this, hepcidin can be considered the fundamental element of the mechanism that lies at the center of the crossroads between natural immunity, responsible for the host immune response, and iron organic homeostasis (22). Finally, other *in vitro* studies have shown that hepcidin in the presence of reduced concentrations of EPO, is able to exert a direct inhibitory effect on the production and on the survival of the erythroid progenitors in the bone marrow (23).

#### ***Reduction of the average life-span of the erythrocytes***

Studies on iron kinetics and erythrokinetics have reported a normal turnover of iron in bone marrow (24, 25) and, at the same time, a modest reduction in the average life-span of the erythrocytes (a hyperhemolytic component) that is not due to intraglobular defects to extra-globular factors (not to anti-erythrocyte antibodies; in fact Coombs tests are negative), but rather to the activation of macrophages by cytokines, and in particular by IL1 and by TNF alpha (26). In

**Table I** - Principle characteristics that differentiate between anemia of chronic diseases and anemia of true iron deficiency.

Laboratory tests	Anemia of chronic diseases (normochromic - normocytic)	Hypochromic-sideropenic anemia (hypochromic-microcytic)
Sideremia	Reduced	Reduced
Total iron binding capacity (TIBC)	Reduced or normal	Increased
Transferrin saturation	Reduced	Reduced
Ferritin	Normal or increased	Reduced
Soluble transferrin receptor	Normal	Increased

any case, it is difficult to imagine that a hyperhemolytic process that is not associated to clear biochemical and hematologic evidence of increased erythrocyte destruction (in fact, bilirubinemia and aptoglobinemia are normal) can be an important cause of anemia. But it is just as difficult to imagine that bone marrow that does not present any great morphological alterations is not able to adequately compensate for such a modest hyperhemolytic process (27).

#### ***Altered proliferative response by the bone marrow erythroid compartment***

It has been suggested that the insufficient compensation of the bone marrow to even modest hyperhemolysis is due to a relative EPO deficiency. Since EPO controls erythropoiesis, in cases of deficiency, its levels would be inadequate for the type and degree of anemia (28, 29). However, there is some discrepancy between the reported results and it has also been hypothesized that there is a reduced hemopoietic response by the erythroid compartment in the bone marrow (30, 31).

In any case, the observation that, in inflammatory pathologies, the degree of anemia correlates with the extent of the underlying phlogistic process (32), and the fact that the improvement or remission of the anemia coincides with that of the primary disease, have led researchers to explore whether the mediators of the immune-phlogistic response could intervene in the pathogenesis of this type of anemia.

Confirmation of this hypothesis is provided by the results of various studies that indicate that cellular and humoral factors would be able to inhibit growth and differentiation of the erythropoietic stem cells (33) both directly (35) and through a reduction in the biological effect of EPO, possibly conditioned by a scarce production of the hormone and by a reduced response on the part of the bone marrow compartment itself (32, 34, 35). In fact, it has been reported that patients with ACD associated to RA have significantly higher levels of IL1- $\alpha$ , IL1- $\beta$ , TNF and IL6 than those with the same disease who are not, however, anemic (31, 32, 36). In particular, as

far as IL6 is concerned, it has been seen that the cytokine levels correlate with those of EPO and that they represent the only inflammatory marker that, independently and inversely correlates with Hb levels (37). Furthermore, it has been reported that administration of human recombinant IL6 in cancers induces the rapid disappearance of dose-dependent progressive anemia that quickly regresses once therapy is suspended (38).

Other studies show that the relative deficiency of the erythropoietin effect on bone marrow is due to proinflammatory cytokines (IL1- $\beta$ , TGF- $\beta$ , IL6) (39, 40). In fact, these cytokines not only suppress EPO production, but would also inhibit the action the hormone exerts on the bone marrow erythroid progenitors (32, 41-43) through a reduction in the hormone receptors present on the cell membrane (42-44). The proinflammatory cytokines exert a direct action on the bone marrow erythroid component inhibiting the proliferation of erythroblastic progenitors. In fact, some authors (32) have observed that the serum of patients with RA or systemic lupus erythematosus (SLE) can inhibit the formation of erythroid colonies in *in vitro* bone marrow cultures (33, 45). Other studies confirm that if bone marrow cultures are obtained in the presence of IL1- $\alpha$  and of IL1- $\beta$  in concentrations similar to those seen in rheumatoid arthritis, there is a clear selective inhibition of the red cell precursors (32, 41). Similar results have been obtained using TFN- $\alpha$  and IL6 (34, 36, 46, 47). In particular, since IL6 is able to promote also hepcidin synthesis (17), it seems to play a double role in ACD pathogenesis, both intervening on metabolic deficiency, resulting in iron deficiency, and on bone marrow, resulting in inefficient erythropoiesis (23, 37).

Finally, the availability of purified cytokines has allowed us to explore the effect of the growth of erythroid progenitors *in vitro* and to show that TFN- $\alpha$  is able to inhibit the growth of human BFUe and CFUe in a dose-dependent manner (46, 47).

In conclusion, based on our present knowledge, it can be seen that the mechanisms responsible for ACD are complex and still

not completely understood. However, it is clear that proinflammatory cytokines play a predominant role in the pathogenesis of this anemia by acting on hepcidin synthesis (with a huge impact on metabolic deficiency) and by its direct or indirect involvement in the proliferation and maturation of the erythroid component within bone marrow.

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