Main Pathophysiological, Diagnostic and Therapeutic Aspects in Anemia of Chronic Inflammation

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ABSTRACT

In chronic inflammation, iron deficiency over time causes a developmental clinical condition ranging from non-anemia to evident microcytic and hypochromic anemia. Pathophysiologically, the iron imbalance in chronic inflammation is mediated by the increase in hepcidin. Its dosage, not yet used routinely, represent an important diagnostic parameter in the setting of anemia, particularly in Anemia of Chronic Deficiency or in the Anemia of Chronic Deficiency/Iron Deficiency Anemia combination. Immunologically, B and T lymphocytes for their activation process need iron to proliferate; in infections, the interaction of myeloid cells, T and B cells, results in inflammatory cytokines secretion which modify iron homeostasis. In chronic inflammation, the rational approach to restore the proper iron flow from the cell to the blood, is to treat the underlying disease, at the same time, adequate iron therapy should be considered. The diagnosis, as well as the correct therapeutic approach to anemia of chronic disease are both increasingly a challenge.

Keywords: Anemia, chronic inflammation, diagnosis, hepcidin, iron therapy.

I. INTRODUCTION

Anemia is a global public health problem affecting about 25% worldwide, 1.62 billion people (Table I) [1].

TABLE I: ANEMIA IN POPULATION GROUP, ITS PREVALENCE AND NUMBER OF AFFECTED POPULATION (SOURCE WHO 2008)

Population group	Prevalence of anemia		Affected population	
	%	95% CI	Number of persons in millions	95% CI
School age children	25,4	19,9-30,9	305	238-371
Pregnant women	41,8	39,9-43,8	56	54-59
Non-pregnant women	30,2	28,7-31,6	468	446-491
Men	12,7	8,6-16,9	260	175-345
Elderly	23,9	18,3-29,4	164	126-202
Total population	24,8	22,9-26,7	1620	1500-1740

Several conditions can cause anemia, one of these is chronic inflammation which, over time, leads to a significant iron imbalance impacting on erythropoiesis process. About 1 billion people worldwide suffer from inflammation/infection anemia (anemia of chronic disease - ACD), which is the most frequent cause of anemia after iron deficiency anemia (IDA) [2]-[4].

In chronic inflammation (CI), iron deficiency causes anemia normochromic and normocytic. Over time, following the progressive and overt hyposideremia, the clinical picture can evolve towards a classic hypochromic and microcytic anemia. Pathophysiologically, in CI iron imbalance is mediated by hepcidin increase (Fig. 1) [5].

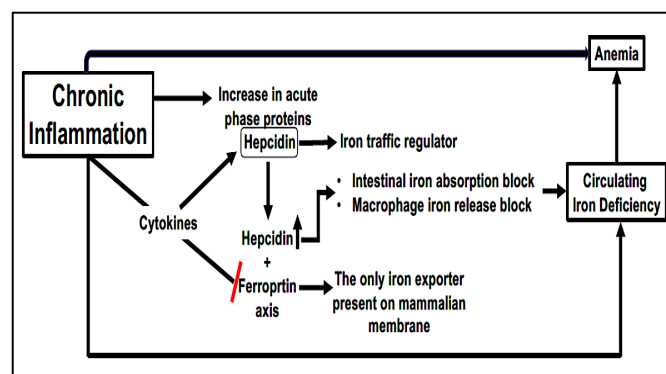The diagnosis, as well as the correct therapeutic approach to ACD are both increasingly a challenge.

Fig. 1. Schematic correlation between inflammation, hepcidin-ferroportin axis, hypoferremia, anemia.

II. IRON DEFICIENCY AND IRON DEFICIENCY ANEMIA

Iron homeostasis process is mainly regulated by (Fig. 2) [6]:

i. Divalent Metatransporter Protein 1 (DMT1), which is the only iron-exporting protein present on mammalian membrane; its function is to facilitate the iron intestinal absorption;

ii. Hephaestin, which, cooperating with Ferroportin, mediates the iron flow from the cells to the blood;

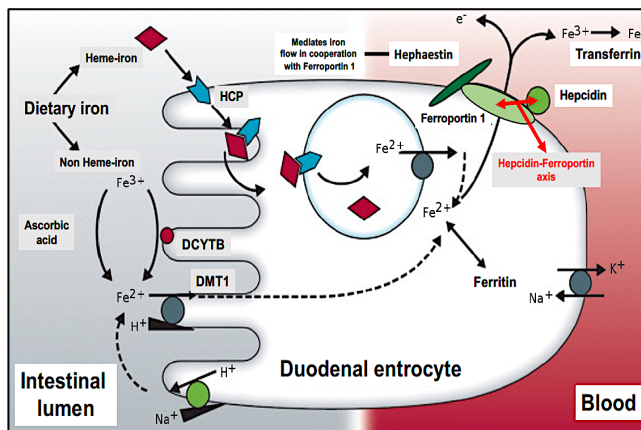iii. Hepcidin-Ferroportin axis, which is the iron homeostasis key point.

Fig. 2. Main pathways of iron metabolism. DCYTB = Duodenal cytochrome B; DMT1 = Divalent Metatransporter Protein 1; HCP = Heme Carrier Protein.

Hepcidin influences the iron flow from the cells to the blood degrading iron transporter ferroportin. Consequently, it effects the plasma iron amount that will bind to the transferrin. Therefore, hepcidin regulates the iron traffic according to the amount of iron required and available.

As part of the iron absorption process, an important role is played by Hypoxia-inducible factor-2 (HIF-2) on DMT1, DYTBC and Ferrportin transcription activation. Additionally, HIF-2 blocks hepatic production of hepcidin [7]. The stabilization of HIF-2 in hypoxia increases the kidney production of erythropoietin (EPO).

Ferroportin-ceruloplasmin cooperation is the preferential pathway to transport iron into the circulation [8]. Intracellular iron which is not exported in blood represents the storage iron (ferritin).

In ID, iron can be recovered through the ferritinophagy process. It is mediated by the nuclear receptor coactivator 4 (NCOA4), a protein that after being bound to ferritin, degrades it [9]. Furthermore, erythroferrone, which is produced by erythroblasts under EPO stimulation, in ID can inhibit hepcidin and promote iron uptake [10].

Diagnostically, it is important to distinguish ID from iron deficiency anemia (IDA). In ID the progressive depletion of stored iron causes its inadequate availability to satisfy the physiological needs. The evolutionary clinical picture of ID is characterized by a transition condition from a non-anemia to an overt anemia. When the stored iron begins to decrease, an early symptomatology, characterized by fatigue, intolerance to physical exercise, reduced cognitive performance can arise. It is essential to contextualize this symptomatology in a framework of incipient hyposideremia.

The Table II shows the main diseases which cause reduced iron absorption and those that cause increased iron losses.

Clinical evolution of advanced ID is an overt IDA. In overt IDA the full blood count shows both MCH and MCV decrease, < 28 pg and < 80 fL respectively, as well as reticulocyte hemoglobin, < 29 pg, whereas the hypochromic red blood cells percentage, a parameter that is provided directly by some blood counters, is increased, > 6%. The MCV and MCH parameters for ID screening are not always reliable because their decrease may not be highlighted in chronic liver, kidney diseases and thalassemia trait. In case of unexplained anemia, IDA is the first cause to consider.

In ID, transferrin saturation (TSAT) is another important parameter to consider. Since both in normal subjects and patients with bone marrow iron deficiency, ferritin can show normal values (50 - 500 µg/L), TSAT and ferritin evaluation allows as to identify patients who benefit from martial therapy. An arbitrary TSAT value < 20% identifies subjects with ID. TSAT value between 20% - 45% indicates adequate iron stores in most patients with CI [11].

Soluble transferrin receptor (sTfR) evaluation in CI can be a useful marker for iron assessment because it is directly related to the erythropoietic bone marrow expansion [12]. Since in CI sTfR can also be affected by cytokines, sTfR/log ferritin ratio is more effective in identifying iron deficiency [13].

TABLE II : MAJOR DISEASES THAT CAUSE REDUCED ABSORPTION AND LOSS OF IRON

Pathologies	Reduced iron absorption	Increased iron losses
Chronic kidney disease	Anorexia/gastrointestinal (GI) tract edema; frequent use of proton pump inhibitors; use of phosphate binders; high hepcidin with intestinal absorption block	Renal uremic syndrome and platelet dysfunction, antiaggregant and anticoagulant therapy; blood loss due to hemodialysis
Heart failure	Anorexia/GI tract edema; high hepcidin with intestinal absorption block	Antiaggregant and anticoagulant therapy
Inflammatory bowel disease	High hepcidin with intestinal absorption block	Chronic diarrhea with high epithelial turnover; GI tract bleeding; corticosteroids therapy
Obesity	High hepcidin due to adipose tissue increase (inflammatory condition); bariatric surgery	Major uterine bleeding (when associated with polycystic ovary syndrome)
Liver disease	Anorexia/GI tract edema; diarrhea caused by laxatives	Bleeding esophageal varices, thrombocytopenia; coagulopathy
Rheumatological disorders	High hepcidin with intestinal absorption block	Corticosteroids and non-steroidal anti-inflammatory drugs therapy

A. Functional Iron Deficiency and Absolute Iron Deficiency

In functional iron deficiency (FID) iron reserves are inadequate, for that reason an imbalance between iron required and serum iron available occurs (Fig. 3) [14].
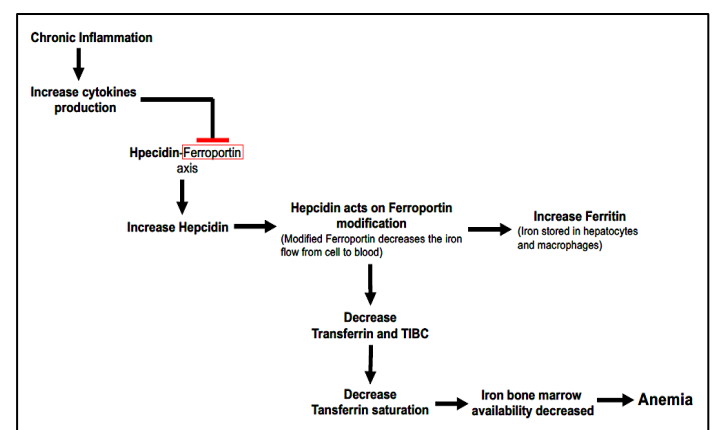

Fig. 3. Functional iron deficiency and anemia.

TABLE III: TREND OF IRON DEPLETION OVER TIME. COMPARISON BETWEEN MAIN FULL BLOOD COUNT INDICES AND TISSUE IRON STORAGE (PERL'S STAINING)

Test	Normal	Iron depletion	Prelatent iron deficiency	Latent iron deficiency	Erythropoiesis in hypoferremia	Early iron deficiency anemia	Late iron deficiency anemia
Serum Ferritin (µg/L)	60	20	< 12	< 12	< 12	< 12	< 12
Iron tissue (Perls) (0 - 4+)	2+	1+	0	0	0	0	0
Transferrin Saturation (%)	35	35	35	20	< 16	< 16	< 16
Hemoglobin (g/L)	140	140	140	140	130	< 120	< 120
MCV (fL)	90	90	90	90	88	88	< 82
MCHC (g/L)	33	33	33	33	33	31	< 28

In absolute iron deficiency (AID) the iron storage is reduced. Consequently, the intra-hepatic transferrin is increased, while transferrin iron-bound in circulation is decreased (Fig. 4) [14]. From FID to AID stage the depletion of stored iron occurs gradually, for this reason the evolutionary aspects of hypoferremia must be evaluated over time (Table III).

Perl's staining on bone marrow aspirate is the gold standard for assessing tissue iron storage. The test, in addition to being invasive, has limited diagnostic advantage [15].
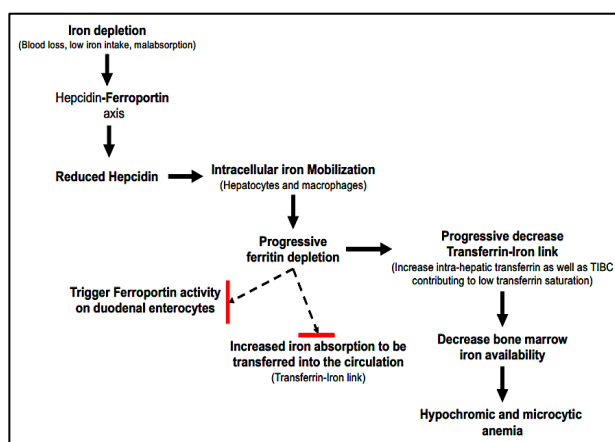

Fig. 4. Absolute iron deficiency and anemia.

III. SERUM INFLAMMATORY MARKERS

The main routine serum markers indicating an inflammatory condition are erythrocyte sedimentation rate (ESR), C reactive protein (CRP) and α-1-glycoprotein (AGP) [16].

The ESR test is affected by hematocrit, fibrinogen and immunoglobulins, the latter for several days remain in circulation [17].

CRP, a protein produced by the liver in response to cytokines, principally IL-6, has a short half-life. CRP value > 50 mg/L in bacterial infections is frequent; it is an excellent acute inflammation marker.

AGP test is suitable for confirming inflammation in chronic phase.

A. Ferritin in Chronic Inflammation

Ferritin is an intracellular iron-binding protein. Its main function is to ensure that the intracellular iron is included in enzymes and hemoglobin, moreover, it prevents the reactive oxygen species (ROS) generation [18], [19].

In addition to being a body's iron stores indicator, ferritin is also an acute phase protein, for that reason its dosage in ACD is inaccurate [20]. According to WHO [21], in adults a ferritin value < 15 µg/L indicates an AID condition.

Moreover, a ferritin threshold value equal or below 30 µg/L has sensitivity and specificity of ~ 92% and ~ 98% respectively in identifying patients with ID. In CI, for CRP values > 80 mg/L, compared to patients with CRP < 10 mg/L, serum ferritin is increased 5-fold. There is general consensus to recommend inappropriately a ferritin cut-off value of 30 µg/L to identify patients with ID in CI.

IV. CHRONIC INFLAMMATION AND HEPCIDIN

When in the early 2000s hepcidin and hepcidin-ferroportin regulatory axis were discovered, the knowledge about iron homeostasis has been completely revolutionized [22]. The iron flow from the cells to the blood is regulated by hepcidin which by binding to ferroportin determines its internalization. Hepcidin, synthesized by the liver, through its ferroportin-degrading action acts as an iron-exporter regulator in blood [23].

Inflammation stimulates macrophages to produce IL-6 which binds to its receptor, IL-6R [24], [25]. Hepatocytes upregulate hepcidin production through IL-6R-JAK2-STAT3 pathway activation; this process occurs in association with bone morphogenetic protein (BMP) 2 and 6-SMAD ligands pathway activation (Fig. 6) [26].

Hepcidin, generally, increases in various chronic inflammatory diseases (rheumatological conditions, inflammatory bowel disease, infections, multiple myeloma, non-Hodgkin lymphoma and critical illness), but in case of CI with simultaneous presence of ACD/IDA, its increase is inhibited by hyposideremia. In these cases, hepcidin dosage can have diagnostic value because it allows us to distinguish pure ACD from the ACD/IDA combination.
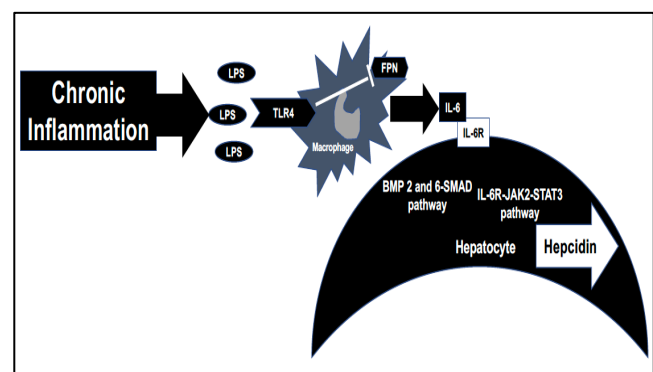

Fig. 5. Schematic hepcidin regulation in chronic inflammation. LPS = Lipopolysaccharide; TLR4 = Toll-like-receptor 4; FPN = Ferroportin; IL-6 = Interleukin-6; IL-6R = Interleukin-6 Receptor.

V. IRON DEFICIENCY AND ADAPTIVE IMMUNITY

Iron is essential for the growth of every living organism.

Its reduced availability in CI, including neoplasms in which inflammation is an intrinsic process, could also be considered a defense to fight infections and tumors. In geographic areas where infections are endemic there is a high incidence of ID.

In infections, the interaction of myeloid cells, T and B cells, results in several inflammatory cytokines secretion, including TNF, IL-1, IL-6, IL-10 and IL-2, which modify iron homeostasis. B and T lymphocytes for their activation process need iron to proliferate [26].

Adaptive immunity studies performed on iron-deficient mice, with or without anemia, which were infected with influenza virus, have shown high sTfR levels in serum and increased TfR expression on activated T lymphocytes, in conjunction with ferroportin inactivation. In this way, the virus recovers and maintains the intracellular iron which is essential for its growth [27]. In SARS-CoV-2 patients, iron deficiency affects both severity and prognosis of disease, moreover, hypoxemia correlates with lymphopenia [28]. The iron/immune response relationship is still a broad field to explore.

VI. THERAPEUTIC APPROACH TO IRON DEFICIENCY IN CHRONIC INFLAMMATION

In CI, the rational approach to restore the proper iron flow from the cell to the blood, is to treat the underlying disease that generates the increase of inflammation-induced hepcidin (Fig. 5). At the same time, adequate iron therapy should be considered (Fig. 6) [29].
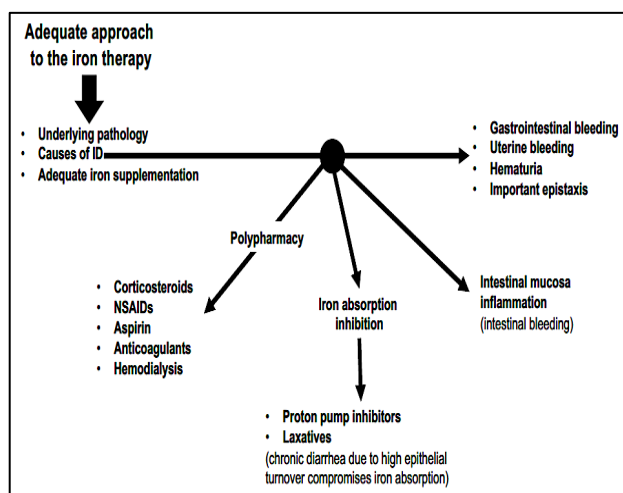

Fig. 6. Iron deficiency management in CI conditions. NSAIDs = Non-steroidal anti-inflammatory drugs.

Iron supplementation can be oral or intravenous (IV). Iron supplementation must take into account the patient's preferences, but mainly the coexistence of comorbidities (Fig. 7).

Oral iron may be the first therapeutic choice because it is cheap and readily available. It should be underlined that oral iron therapy in 30% - 70% of patients causes gastrointestinal intolerance, resulting in a lack of adherence to therapy.

IV iron therapy makes iron more readily available than oral therapy. Patients with inflammatory bowel disease, heart failure, chronic kidney disease or perioperative anemia should be treated with IV iron. In addition, IV iron therapy must be implemented in patients with gastrointestinal tract

resection (including bariatric surgery), prolonged use of proton pump inhibitors and gastrointestinal intolerance to oral iron.

IV iron has side effects at the injection site or it can cause potentially fatal hypersensitivity. For these reasons, therapy should be carried out in a hospital setting. High-doses therapy with less stable iron complexes can trigger oxidative stress. IV iron therapy is more expensive than oral iron.
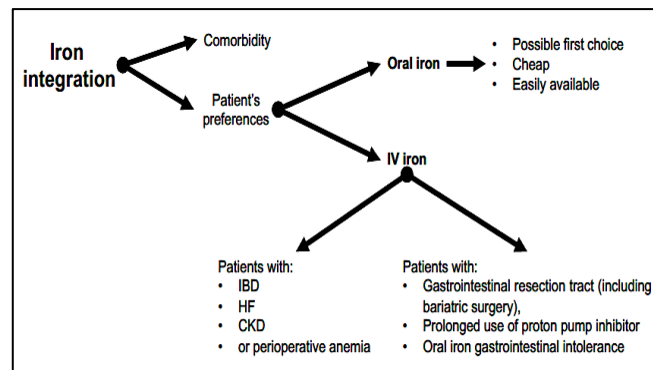

Fig. 7. Approach to the iron therapy. IBD = inflammatory bowel disease; HF = heart failure; CKD = chronic kidney disease.

Erythropoiesis-stimulating-agents (ESA) therapy is another option in ACD [30]. EPO therapy promotes the entry of Burst Forming Unit-Erythroid (BFU-E) into the active cellular cycle, preventing Colony Forming Unit-Erythroid (CFU-E) apoptosis, increasing globin chains synthesis as well as the transferrin receptor (TFR) expression on cells surface. EPO therapy:
  i. is a physiological treatment;
 ii. can be carried out at home;
iii. improves quality of life (QoL); iv) has an excellent tolerance.

EPO therapy has the disadvantage of being effective only in a fraction of patients and it work after weeks. Moreover, the therapeutic path is expensive.

Experimental therapeutic approaches are aimed at controlling the hepcidin-ferroportin axis. Hepcidin agonists in iron overload diseases, could have a therapeutic rationale contributing to the improvement of low hepcidin levels [31]. Hepcidin antagonists could instead be employed in ACD and Iron Refractory Iron Deficiency Anemia (IRIDA) in order to release the intracellular trapped iron [32].

In anemia nutritional status also plays an important role. A normal erythropoietic process needs not only iron, but also vitamin B12, folic acid, vitamin C, vitamin B6, hormones, proteins. In multifactorial anemia, any missing substances aforementioned should be supplemented [33].

Iron therapy monitoring effects is still based on empirical hemoglobin increase of at least 1 g/dL after 1 month from the start of oral therapy. If not, the patient is considered refractory to oral iron supplementation and could be candidate for IV iron therapy. After 2-3 months of oral iron therapy, empirically for ferritin parameter reaching levels of at least 100 mg/L an adequate iron storage restoration can be considered. If not, it is advisable to continue oral iron therapy so that in a short time a relapse of anemia does not occur, such as when the primary cause of anemia is not easily/immediately eliminated (e.g., menorrhagia).

In parenteral iron therapy, the response to the increase in

hemoglobin is much faster than the oral one. However, in these cases ferritin does not represent a reliable parameter for estimating iron storage, since its value is disproportionately high even for several weeks after infusion.

Reticulocyte testing may be appropriate for assessing response to oral iron therapy. However, from the start of therapy, the timing for response assessment is individually variable. In this regard, reticulocyte hemoglobin (CHr) could be an essential additional parameter to consider. CHr is reduced in all conditions in which there is an iron-deficient erythropoiesis; if iron therapy is effective and, consequently, a physiological amount of iron is available again, the CHr value increases within a few days. CHr is automatically measurable by modern blood cell counters and the test is available at no additional cost [34].

VII. CONCLUSIONS

ACD is a very common condition that generally occurs in the elderly with chronic inflammation. It must always be suspected and evaluated in relation to the general clinical picture. It is often associated to with true iron deficiency.

For ACD diagnosis, empirical cut-offs for hemoglobin, ferritin and TSAT are considered. However, a proper combined evaluation of ferritin and TSAT can identify patients who might benefit from iron therapy. Hepcidin dosage represents a significant diagnostic parameter due to its central role in iron homeostasis, but its dosage is not yet a routine test.

For anemia correction, iron therapy must be considered in relation to the underlying disease evolution, as well as to the balance between advantages and disadvantages that it could provide. Among the advantages are the QoL improvement, while same of the disadvantages are the possibility that the contemporary treatments in place could favor immunosuppression and the growth of pathogens.

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