A holistic approach of erythropoiesis and iron in anemia

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Abstract

Background: Iron (Fe) metabolism is dependent on heme biosynthesis in bone marrow erythroblasts and is strictly controlled. Failure of this process induces to absolute or functional iron deficiency anemia (IDA), which is common in general medical practice.
Aim: This study summarizes the Fe role in normal and disturbed erythopoietic process and focuses on current biochemical indicators and available therapeutic options for Fe imbalance.
Methods: A systematic review in PubMed, MedLine and MDConsult database was conducted. The research limits included English abstracts and full texts, referring to erythropoiesis, anemia and Fe during the last decade.
Results: Erythrocyte delivers oxygen to the tissues, so the primary consequence of anemia is tissue hypoxia. Oxygen-sensing cells in kidney respond to hypoxia by increasing erythropoietin (EPO), the basic regulatory hormone of erythropoiesis. Fe is an essential micronutrient for adequate erythropoietic function. Anemia of chronic disease (ACD) and IDA are characterized by disturbances in Fe homeostasis. In addition, inflammatory disorders and renal insufficiency are complicated by severe depression of EPO levels. Red cell indices (hemoglobin, mean cell hemoglobin) and biochemical markers (ferritin, transferrin saturation and receptors) reflect hemoglobin biosynthesis and iron pathways. The therapy of underlying disease is essential in IDA and ACD, whereas adjuvant procedures are red blood cells transfusion, erythropoietic agents and Fe supplementation.
Conclusion: The approach of Fe-deficient patient is complex and based on wide range of clinical and laboratory findings. The precise evaluation of them is critical for diagnosis and management modalities.

Key words: Erythropoiesis, Iron metabolism, Hepcidin, Anemia, Chronic disease

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1.Introduction

Erythropoiesis is a part of the larger process of hemopoiesis, which first appears in the yolk sac at around day 21 of gestation. The multipotent hemopoietic stem cell gives rise to progeny cell lineages that become committed to a single cell type e.g. erythrocytes [1].

Erythroid cells are found in multilineage colonies, CFU-GEMM, which include granulocytes, macrophages and megakaryocytes. The first truly committed erythrocyte progenitor is a cell lineage called burst-forming unit-erythroid (BFU-E). The later stage, colony-forming unit-erythroid (CFU-E), is a more mature cell and differentiates into proerythroblasts and

erythroblasts. The reticulocyte gives rise to the mature enucleated erythrocyte, after one day of circulation in the peripheral blood. Through differentiation nucleoli disappear, nucleus condenses and finally extruded, the cell size is reduced and large amounts of hemoglobin (HGB) are synthesized [2].

A primary function of erythrocytes is to transport oxygen from the lungs to the tissues and organs in the body. This means that red cell mass represents the oxygen-carrying capacity of the body. The normal red blood cell lifespan is 120 days and in a normal adult the daily turnover exceeds 10^{11} cells [3, 4]. The balance, between the loss of mature red cells and new production, is maintained by an oxygen-sensing system, which responds via erythropoietin (EPO) production. Cytokines, growth factors, hormones, interactions with stromal cells in the bone marrow and elements, such as iron (Fe), folate and vitamin B12, are involved in control mechanisms of the process. Abnormalities in any of these factors can affect red cell mass, resulting in anemia or erythrocytosis [5].

EPO is a glycosylated hormone, produced primarily in the kidney peritubular cells, although small amount is produced by the liver. The EPO gene is under the regulation of the hypoxia inducible factor 1α (HIF-1α). During hypoxia the α-subunits of HIF-1 are heterodimerized with HIF-1β and activate the hypoxia-response genes, including EPO gene [6]. EPO interacts with its receptor (EPO Receptor, EPOR) on the surface of erythroid progenitor cells in the marrow. This promotes the activation of several transduction pathways (STAT5, Ras/MAP kinase and PI3 kinase) and the production of red blood cells. The EPOR appears for the first time in BFU-E cells and continued stimulation with EPO triggers differentiation into erythroblasts. Reticulocytes and mature erythrocytes do not express EPOR and are not sensitive to EPO action [3, 4].

2.Iron physiology

The presence of Fe is essential for erythropoiesis, contributing to HGB production at the later stages of erythroid differentiation. Approximately 200 billion new red blood cells are generated daily, requiring 20-25mg of Fe. A normal western diet provides 15mg Fe daily, from which only 1mg (or 5-10% of dietary Fe) is absorbed to the portal blood. The main source of the Fe, used by developing red cells precursors, is from senescent red cells, which are phagocytosed by reticuloendothelial macrophages [3, 4, 7].

Food Fe is found in three forms: ferrous (Fe^{+2}), ferric (Fe^{+3}) and heme. Fe is absorbed in the proximal duodenum by enterocytes. The heme Fe is readily absorbed, whereas ferric Fe is reduced to ferrous before entering to enterocyte. This stage is facilitated by the divalent metal transporter (DMT). In the enterocyte is either incorporated into ferritin and sloughed with aging enterocyte or is transported through basolateral surface of the enterocyte, through a tubular transmembrane protein, ferroportin and eventually enters the bloodstream. Transferrin is a polypeptide, which transports Fe from enterocytes or sites of Fe stores to the developing erythroid cells. The uptake of Fe from transferrin requires the interaction with its receptor (TransFerrin Receptor, TFR) [8, 9, 10].

The hepcidin is a peptide which is predominantly expressed in the liver and has a major role in regulation of Fe absorption in gastrointestinal tract and Fe availability from reticuloendothelial system (RES). Hepcidin exerts its action by binding to ferroportin, causing its downregulation and removal from the cell surface. The Fe status, the inflammation and the erytrhopoiesis itself are regulator factors of hepcidin production [11]. In fact, body's Fe needs and stores control the production of the molecule in a process, which involves the hemochromatosis gene HFE, the TFR2 and the hemojuvelin. Fe overload and inflammation increase hepcidin production, whereas ineffective erythropoiesis and tissue hypoxia cause a decrease in hepcidin synthesis [12, 13].

3. Anemia

Anemia is a syndrome, resulting from a reduced red cell mass more than two standard deviations from the mean for age, gender and race. In many studies the anemia

is defined according to World Health Organization (WHO) criteria (HGB<13gr/dl for male and <12gr/dl for female), suggested nearly 40 years ago. Nowadays, the definition of "the lower limit" of HGB is an issue of interest because of its normal variability and its significance in outcome of several disorders. The anemia is a result of impaired erythrocyte production, increased rate of erythrocyte destruction, immunologic disorders, nutritional deficiencies and a broad spectrum of systemic diseases [14].

The clinical manifestations of anemia are due to impaired tissue oxygenation. Compensatory mechanisms, such as peripheral vasodilation, increased cardiac output, changes in oxygen-HGB dissociation curve and shunting of blood to critical organs (heart and brain), maintain the oxygenation. This explains why the anemia usually is recognized by abnormal laboratory screening tests. Few symptomatic patients usually complain for fatigue, shortness of breath and tachycardia. In severe acute anemia, due to blood loss or hemolysis, patients may be unable to compensate and confusion, air hunger, sweating, hypotension and tachycardia are prominent [15].

In practice, the cause of anemia is not readily apparent and the efforts of further investigations are guided by patient's past medical and family history, clinical features, complete blood count, reticylocyte count (REC), red cell indices (Mean Cell Volume, MCV; Mean Cell HGB, MCH; Mean Cell HGB Concentration, MCHC) and red cell morphology in blood films. More specific tests are conducted according to diagnostic suspicion. These include Fe status, HGB synthesis, folate or vitamin B12 levels, hereditary defects of red cell membrane and enzymes and other hemolytic mechanisms [16].

3.1 Iron Deficiency Anemia (IDA)

The most common anemia worldwide is IDA, which is suspected from the presence of hypochromic and microcytic erythrocytes. The iron deficiency (ID) is a result of disrupted balance between Fe absorption, Fe transport and Fe storage in human body. The three distinct mechanisms are: (a) increased Fe requirements (e.g. use of rhEPO, pregnancy, and post-bleeding recovery), (b) defective Fe intake (e.g. malnutrition) or malabsorption (e.g. inflammatory bowel disease) and (c) increased blood loss (e.g. menorrhagia, hemorrhoids, and peptic ulceration and bowel tumors). Absolute ID is a term, used in case of depleted Fe stores. In functional ID, Fe stores are replete, but cannot be mobilized as fast as necessary from the RES to the bone marrow (e.g. anemia of inflammatory disease) [10]. The definitive diagnosis of IDA highlights the need of causative investigation.

Diagnostic methods for Fe status assessment include: (a) Fe stores (ferritin), (b) tissue Fe supply (serum Fe; total iron binding capacity, TIBC; transferrin saturation, TSAT; soluble TFR, sTFR), (c) functional Fe (HGB, MCV, MCH). In clinical practice, a combination of laboratory parameters is usually required, since no single measurement is ideal for all cases.

Normal HGB level does not exclude ID. Increased Fe absorption and mobilization from body's stores occur in a state of negative Fe balance. The main laboratory finding is low ferritin level in absence of inflammation (CRP<0.5 mg/dl), whereas serum Fe, MCV and MCH are still normal. Ferritin belongs to acute phase reactant proteins, so in inflammatory disorders normal ferritin level does not exclude ID. In this case, the investigation includes TSAT, which reflects the Fe availability to erythroid precursors.

In frank IDA the patient presents with low HGB, ferritin and serum Fe, serum TIBC rises, TSAT is usually less than 20%, MCV and MCH are reduced. The absence of microcytosis is possible, when vitamin B12 or folate deficiency coexists. The REC is low for the degree of anemia. The levels of soluble TFR (sTFR) are usually high, but rarely used for diagnosis of uncomplicated IDA [2, 17].

3.2 Anemia of chronic disease (ACD)

Anemia is a frequent complication in systemic diseases (e.g. chronic infections, chronic inflammatory disorders, malignant diseases, congestive heart failure and chronic renal failure). Activation of immune mechanisms results in impaired red cells lifespan and proliferation, relative low levels

of EPO, reduced response of erythroid cells to EPO and disturbances of Fe homeostasis [8].

This type of anemia is usually normochromic or mildly hypochromic and is characterized by low serum Fe with adequate RES stores and reduction of Fe granules in marrow erythroblasts. Hepcidin levels are up-regulated in inflammation by several cytokines, including interleukin 6 (IL-6), IL-1 and tumor necrosis factor α (TNF-α). Hepcidin binds to ferroportin and leads to its internalization and degradation. This decreases Fe absorption in gut and blunts Fe mobilization from store sites in hypatocytes and macrophages.

The laboratory findings include high CRP levels, HGB<13gr/dl for males and <12gr/dl for females, TSAT<20%, normal or high serum ferritin concentration and sTFR/log ferritin ratio<1. In case of ID coexistence, serum ferritin concentration is lower than 100ng/ml and sTFR/logferritin ratio>2 [17, 18, 19]. Measurement of intrinsic EPO levels is useful in cases with HGB< 10g/dL, because in mild anemia the EPO remains in normal range [20].

With further validation, hepcidin may be used as a marker for diagnosis of ACD with or without ID. Patients with ID co-existence have lower hepcidin level, than those with normal Fe values, maintaining the ability of dietary Fe absorption and mobilization from macrophages [21, 22].

3.3 Anemia of chronic renal disease (ACRD)

Patients with acute or chronic failure develop anemia, which is associated with the loss of normal inverse linear relation between plasma EPO and HGB concentration, the existence of erythropoietic, diminished red cell survival, the deficiency of Fe and folate in dialysis patients. In acute conditions, the anemia is strongly related with the causative factor e.g. sepsis, microangiopathy [2, 23].

Anemia is normochromic and normocytic with the presence of ecchinocytes in the blood film. The REC is normal or slightly low and the bone marrow shows normoblastic erythropoiesis.

Recombinant human EPO (rhEPO) is used systematically for ACRD. Concomitant Fe therapy is usually required, due to ID or impaired mobilization from store sites. Deficiency of Fe, cobalamin or folate, hemolysis, infections, occult malignancies, aluminium toxicity and hyperparathyroidism are the main causes of reduced response to EPO therapy [23].

4. Treatment options

Treatment of the underlying disease is the gold standard in anemia with Fe disturbances. Many studies show improvement in HGB level only by the limitation of bleeding sites and reduction of harmful cytokine levels (24). Nevertheless, when this strategy is not feasible, alternative supportive care is necessary.

4.1 Transfusion medicine

Transfusion of red blood cells is used as a rapid and effective intervention in cases of severe anemia (HGB<7gr/dL), active bleeding, and life-threaten comorbidities (e.g. coronary disease, heart and respiratory failure).

Generally, long-term transfusion practice is not recommended in anemic patients with Fe imbalance, because of transfusion-associated complications, such as viral infections, sensitization, febrile reactions and Fe overload.

4.2 Erythropoiesis Stimulating Agents

Therapy with rhEPO has been used successfully to treat ACRD, ACD and anemia associated with cancer and AIDS. The improvement of performance status and quality of life in these situations is obvious.

Nowadays, there are several erythropoietic agents with different circulating half-life according to the pattern of glycosylation or pegylation of EPO molecule. Longer terminal half-life permits the administration of erythropoietic agents in more extended intervals [3]. Several clinical studies have investigated different dosing schedules (once weekly, once every two or three weeks and once monthly) across different clinical setting [25]. Notably, in patients with myelodysplastic syndrome, endogenous EPO level less than 200u/L correlates with better response rate.

Few data are available concerning possible adverse effects of long-term

erythropoietin administration on the course of underlying chronic disease [26], due to the expression of EPOR in some malignant cell populations [27]. For example, the drug led to the myeloma regression in murine models, whereas promoted the proliferation in human renal carcinoma cells [28, 29].

4.3 Iron supplementation

Ferrous sulfate and ferrous gluconate, the most common oral preparations, have been used in IDA in the recommended dose of 150mg of elemental Fe daily. The responders increase the HGB level 1gr/dL within about two weeks. Treatment should be continued for two to three months after anemia correction, to fulfill Fe stores [30]. Poor absorption or noncompliance highlights the need of parenteral Fe administration. Common parenteral agents are Fe sucrose (VENOFER®), Fe dextran (COSMOFER®) and more recent ferric carboxymaltose (FERRINJECT®).

Supplementation with Fe agents is controversial in ACD, because Fe is an essential nutrient for microorganisms and tumor cells [31]. In addition, in some chronic diseases (e.g. rheumatoid arthritis) Fe therapy is implicated in the formation of toxic radicals. Nevertheless, Fe supplementation is recommended in patients with absolute ID and in those receiving erythropoietic agents with poor response due to the developing functional ID [32].

Conclusion

Erythropoiesis and Fe homeostasis are tightly controlled processes resulting to red cell production. Fe is an essential metal micronutrient and is distributed mainly within erythrocytes HGB. Conditions, that disturb this process and the balance between red cell production and destruction, provoke anemia. Anemia is an indicative syndrome of an underlying disease. The commonest type of anemia worldwide is IDA, which usually complicates ACD. In the absence of inflammation, ferritin alone provides the diagnosis of absolute ID. In ACD, the combined use of laboratory tests, such as ferritin, TSAT and sTFR, is mandatory for evaluation Fe status. Therapeutic modalities are conducted according to clinical and laboratory findings, ranged between causative management and indicative care.

Bibliography

1. Palis J, Robertson S, Kennedy M, Wall C, Keller G. Development of erythroid and myeloid progenitors in the yolk sac and embryo proper of the mouse. Development. 1999; 126 (22): 5073-5084
2. Hoffbrand A, Catovsky D, Tuddenham E. Postgraduate Haematology. 5th ed. Blackwell Publishing; UK, London; 2005
3. Elliott S, Pham E, Macdougall IC. Erythropoietins: A common mechanism of action. Experimental Hematology. 2008; 36 (1): 1573–1584
4. Walter Fried. Erythropoietin and erythropoiesis. Experimental Hematology. 2009;37(9): 1007-1015
5. Herbert Y. Lin. Erythropoiesis: The Roles of Erythropoietin and Iron. In: Singh AK, Williams GH, editors. Textbook of Nephro-Endocrinology. 2nd ed. Elsevier; USA; 2009
6. Ivan M, Kondo K, Yang H, Kim W, Valiando J, Ohh M, et al. HIF alpha targeted for VHL-mediate destruction by praline hydroxylation: implications for O_2 sensing. Science. 2001; 292 (5516): 464-468
7. Nadadur S, Srirama K, Mudipalli A. Iron transport & homeostasis mechanisms: Their role in health & disease. Ind J Med Res. 2008; 128 (4): 533-544
8. Iolascon A, Falco L, Beaumont C. Molecular basis of inherited microcytic anemia due to defects in iron acquisition or heme synthesis. Haematologica. 2009; 94 (3): 395-408
9. Bleackley MR, Wong AY, Hudson DM, Wu CH, Macgillivray RT. Blood iron homeostasis: newly discovered proteins and iron imbalance. Transf Med Rev. 2009;23 (2): 102-123
10. Morón C, Viteri F. Update on common indicators of nutritional status: food access, food consumption, and biochemical measures of iron and anemia. Nutrition Reviews. 2009; 67 (1): 31–35

11. Piperno A, Mariani R, Trombini P, Girelli D. Hepcidin modulation in human diseases: From research to Clinic. World J Gastroenterol. 2009; 15 (5): 538-551
12. Fleming RE, Bacon BR. Orchestration of iron homeostasis. N Engl J Med. 2005; 352 (17): 1741-1744
13. Haurani FL. Hepcidin and the anemia of chronic disease. Ann Clin Lab Sci. 2006; 36 (1): 3-6
14. Beutler E, Waalen J. The definition of anemia: what is the lower limit of normal of the blood hemoglobin concentration? Blood. 2006; 107 (5): 1747-1750
15. Burnett D, Blair C, Haeney MR, Jeffcoate SL, Scott KW, Williams DL. Clinical pathology accreditation standards for the medical laboratory. Journal of Clin Path. 2002; 55 (10): 729-733
16. Goldman: Cecil Medicine. 23rd ed. Elsevier, USA; 2007
17. Muñoz M, Villar I, García-Erce J. An update on iron physiology. World J Gastroenterol. 2009;15 (37): 4617-4626
18. Roy CN, Andrews NC. Anemia of inflammation: the hepcidin link. Curr Opin Hematol. 2005; 12 (4): 107-111
19. Weiss G, Goodnough LT. N Engl J Med. 2005; 352 (10): 1011-1023
20. Miller CB, Jones RJ, Piantadosi S, Abeloff MD,M Spivak JL. Decreased erythropoietin response in patients with the anemia of cancer. N Engl J Med. 1990; 322 (24):1689-1692
21. Yang B, Zaritsky J. Hepcidine for Clinicians. Clin J Am Nephrol. 2009; 4 (8): 1384-1387
22. Theurl I, Aigner E, Theurl M, Nairz M, Seifert M, Schroll A, et al. Regulation of iron homeostasis in anemia of chronic disease and iron deficiency anemia: diagnostic and therapeutic implications. Blood. 2009; 113 (21): 5277-5286
23. Brenner and Rector's. The kidney. 8th ed. Saunders Elsevier, USA; 2007
24. Weiss G. Pathogenesis and treatment of anemia of chronic disease. Blood Rev. 2003; 16 (11): 87-106
25. Macdougall IC. Novel Erythropoiesis-Stimulating Agents: A new Era in Anemia Management. Clin J Am Soc Nephrol. 2008; 3(1): 200-207
26. Jelkemann W. Proinflammatory cytokines lowering erythropoietin production. J Inter Cyto Res. 1998; 18 (2): 555-559
27. Acrasoy MO, Amin K, Karayal AF, Chou SC, Raleigh JA, Varia MA, et al. Functional significance of erythropoietin receptor expression in breast cancer. Lab Invest. 2002; 8 (7): 911-918
28. Mittelmann M, Neumann D, Peled A, Kanter P, Haran-Ghera N. Erythropoietin induces tumor regression and antitumor immune responses in murine myeloma models. Proc Natl Acad Sci USA. 2001; 98 (9): 5181-5186
29. Westenfelder C, Baranowski RL. Erythropoietin stimulates proliferation of human renal carcinoma cells. Kidney Int. 2000;58 (2): 647-657
30. Rakel. Text Book of Internal Medicine. 7th ed. Saunders Elsevier,USA; 2007
31. Weinberg ED. Iron loading and disease surveillance. Emerg Infect Dis. 1999; 5 (3): 346-352
32. Auerbach M, Ballard H, Trout JR, Mcllwain M, Ackerman A, Bahrain H, et al. Intravenous iron optimizes the response to recombinant Human erythropoietin in cancer patients with chemotherapy–related anemia: a multicenter, open-label, randomized trial. J Clin Oncol. 2004; 22 (7): 1301-1307