Iron overload in hematological disorders

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Summary

While most common symptom of impairment of iron homeostasis is iron deficiency anemia, some hematological disorders are associated with iron overload (IO). These disorders are related mainly to chronic severe hemolytic anemia, where red blood cells (RBC) or their precursors are destroyed prematurely (hemolyzed), leading to anemia that cannot be compensated by increased production of new RBC. In such cases, IO is mainly due to repeated RBC transfusions and/or increased uptake of iron in the gastrointestinal tract. Normally, iron is present in the plasma and in the cells bound to compounds that render it redox inactive. Iron overload leaves a fraction of the iron free (labile iron pool) and redox active, leading to the generation of excess free radicals such as the reactive oxygen species. This condition upsets the cellular redox balance between oxidants and antioxidants, leading to oxidative stress. The free radicals bind to various cellular components, thereby becoming toxic to vital organs. Oxidative stress may also affect blood cells, such as RBC, platelets and neutrophils, exacerbating the anemia, and causing recurrent infections and thrombotic events, respectively. The toxic effect of IO can be decreased by treating the patients with iron chelators that enter cells, bind free iron and remove it from the body through the urine and feces. Iron toxicity may be also ameliorated by treatment with anti-oxidants that scavenge free radicals and/or correct their damage. The use of iron chelators is widely accepted when started in young patients with severe chronic anemia, but is still debatable as a therapeutic modality for older patients suffering from IO due to myelodysplastic syndromes. It should be noted that in addition to preventing iron toxicity, some compounds with iron chelator activity may also benefit other aspects of hematological disorders. These aspects include stimulation of platelet production, inhibition of leukemic cell proliferation and induction of their differentiation. Compounds with such multiple activities may prove beneficial for at least some patients with leukemia and myelodysplastic syndromes.
where developing erythroid precursors undergo enhanced apoptosis (ineffective erythropoiesis); (b) in the reticuloendothelial system, mainly in the spleen and liver, where senescent RBC undergo phagocytosis by resident macrophages (extravascular hemolysis); and (c) in the peripheral blood, where pathological RBC undergo lysis in the blood vessels (intravascular hemolysis). As a result of these processes, RBC destruction exceeds the ability of the body to supply new ones, resulting in chronic anemia. The causes of IO in these conditions are an accumulation of iron due to repeated blood transfusions given in order to ameliorate the chronic anemia, and increased absorption of nutritional iron from in gastrointestinal (GI) tract. Since the body lacks an efficient mechanism of getting rid of excess iron, it is accumulated in multiple organs, including vital ones such as the heart and liver, leading to their malfunctioning. Regardless its source, the damage caused by IO is mediated by free radicals, such as reactive oxygen species (ROS) [1]. Free iron, and especially its cellular redox-active form, the labile iron pool (LIP), catalyzes the generation of ROS, leading to oxidative stress with its consequential cytotoxicity [2]. This review will focus on the involvement of IO in cases other than gene mutations associated with hereditary hemochromatosis. Although IO is not the primary etiology of the hematological disorders that will be discussed in this chapter, it is a major cause of morbidity and mortality in this group of patients [1]. The treatment of IO relies mainly on the use of iron chelators that can enter cells, bind access iron and remove it from the body. The involvement of ROS in iron-induced toxicity may suggest the therapeutic use of antioxidants as well [3]. The deleterious effects of iron and ROS extend beyond their cytotoxicity; they may be involved in additional pathological aspects such as induction of genetic instability leading to malignant transformation, stimulation of malignant cell proliferation and inhibition of their differentiation [4]. Although iron chelation therapy has made tremendous progress in recent decades in treating hematological patients, extending its indications and developing drugs of multiple targets is still required.

Hematological disorders associated with iron overload

The hereditary anemias, either hereditary or acquired, are the main hematological disorders associated with IO. The hereditary anemias are due to mutations in the globin genes (hemoglobinopathies) or in other genes affecting the production and survival of RBC. The acquired hemolytic anemias are included mainly in the myelodysplastic syndrome (MDS) and the myeloproliferative diseases (MPD).

Hereditary hemolytic anemias

Thalassemias

One of the most frequent hereditary hemolytic anemia worldwide. Their pathophysiology is due to a partial or complete deficiency in the synthesis of α- or β-globin chains that compose the Adult hemoglobin (HbA), a tetramer of α2β2, due to several hundred possible mutations in their corresponding genes. The unpaired globin chains are unstable; they precipitate intracellularly, resulting in the premature destruction of the RBC precursors in the bone marrow and extravascular hemolysis of the mature RBC. The breakdown products of Hb, heme and iron, increase oxidative stress by generating oxygen free radicals. Beta-thalassemia is classified into three main forms based on the clinical severity: major, intermedia, and minor. Individuals with β-thal major usually present within the first 2 years of life with severe anemia, poor growth, and skeletal abnormalities, and they require regular, lifelong, blood transfusions. β-thal intermedia may require only episodic blood transfusions. Alpha thalassemia results in moderate hemolytic anemia when there is a significant lack of synthesis of α-globin chains (HbH disease). Iron overload constitutes a severe pathology in most cases of thalassemia [5].

Sickle cell disease (SCD)

In SCD, a specific point mutation (GAT→GTT) in the sixth codon of exon-1 of the β-globin gene results in the production of abnormal β-globin chains (βS) that bind to α-globins to produce Sickle Hb (HbS). When the RBC experience deoxygenating conditions (like those that occur in the blood capillaries), HbS undergoes polymerization. This causes changes in the physical and chemical properties of the RBC, which result in sickle-like shape and hemolysis as well as increased tendency to adhere to the vasculature walls [6]. Sickled RBC blocking of the blood flow to the limbs and organs may cause pain and organ damage (sickle cell crisis). The symptoms in SCD are related to the anemia, sickle cell crisis, and their complications such as acute chest syndrome, pulmonary hypertension, and stroke. Some patients are treated by regular RBC transfusion, but since the treatment causes IO, it is difficult to assess to what degree organ damage is related to the disease itself or the IO caused by the RBC transfusions [7].

Glucose-6-phosphate dehydrogenase (G6PD) deficiency

It is caused mainly by point mutations in the gene coding for G6PD, a key enzyme of the pentose pathway (hexose monophosphate shunt), which is essential for an adequate supply of nicotinamide adenine dinucleotide phosphate (NADPH) – a reducing agent that is important for several cellular biosynthetic pathways [8] and for monitoring of cellular redox regulation [9]. RBC have limited reducing power, and in the absence of intracellular organelles, they depend solely on the pentose pathway for the generation of NADPH. Increased oxidative stress in G6PD-deficient RBC is well documented [10]. Individuals with the disease may exhibit nonimmune hemolytic anemia in response to a number of causes, most commonly infection or exposure to certain medications or chemicals and nutrients (e.g., fava beans). The disease is often associated with IO [11].

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**Hereditary spherocytosis**
It is characterized by RBC that are sphere-shaped (rather than biconcave disks), which reduce their surface to volume ratio and increase their osmotic fragility, properties which make them more prone to hemolysis. It is caused by mutations in genes for a variety of membrane proteins. Spectrin deficiency is the most common abnormality [12], but in some cases, it is secondary to a deficiency or dysfunction of the spectrin-binding protein ankyrin [13]. The hemolysis results in varying degrees of anemia. Iron overload was reported in some cases of this disease [14].

**Congenital dyserythropoietic anemias (CDA)**
A heterogeneous group of rare diseases in which the anemia is predominantly caused by ineffective erythropoiesis and distinct morphological abnormalities of erythroid precursor cells in the bone marrow (dyserythropoiesis). Three major (types I, II and III) and several minor subgroups have been identified [15]. The gene responsible for CDA1 was identified as CDAN1, encoding codanin-1 [16]. Iron overload develops with age as a result of increased iron absorption even in those who are not transfused [17].

**Acquired hemolytic anemias**

**Paroxysmal nocturnal hemoglobulinuria (PNH)**
A rare clonal disorder caused by an acquired somatic mutation predominantly in the phosphatidylinositol glycan complementation class A (PIG-A) gene located on the X-chromosome at Xp22.1. This gene encodes the enzyme responsible for the first step in the production of the glycosylphosphatidylinositol (GPI) anchor, by which various proteins are linked to the plasma membrane. When these mutations occur in a hematopoietic multipotent stem cell, there is a partial or complete deficiency of GPI-anchored proteins on the surface of the stem cell and its progeny. These mutated cells remain an inconsequential minority within the hematopoietic cell population, until some unknown conditions (e.g., autoimmune features) provide them with a proliferative/survival advantage over the non-mutated cells, thus, generating considerable subpopulations of mutated hematopoietic cells of various lineages [18]. The disease is characterized mainly by intravascular hemolysis, which is due to hyper-sensitivity of the affected RBC to activated serum complement. This is due to their lack/deficiency of CD55 (decay-accelerating factor), which inhibits complement at the level of C3, and CD59 (membrane inhibitor of reactive lysis), which prevents terminal complement components (C5b-9) from forming the hemolytic membrane pore [19]. Other symptoms include frequent infections, bone marrow hypoplasia and cytopenia, and venous thrombosis [20]. Iron overload may develop depending on the number of transfusions [21].

**Autoimmune hemolytic anemia**
It is caused by auto-antibodies against antigens expressed on the surface of RBC. Once formed, these antibodies bind to the surface of RBC, marking them for destruction through complement-mediated lysis (intravascular hemolysis) and/or Fc-mediated phagocytosis (extravascular hemolysis). Autoimmune hemolytic anemia can occur alone but is most often seen in association with other autoimmune diseases, cancer, drug treatment, transfusion, and pregnancy [22]. Depending on the load of transfusions, IO may develop [23].

**Myelodysplastic syndrome (MDS)**
It is due to the ineffective production (dysplasia) of hematopoietic cells in the bone marrow. There are several forms of MDS—from refractory anemia to pancytopenia—that within months to a few years transform to acute myeloid leukemia in about a third of the patients. Iron overload in MDS is related to the load of RBC transfusions [24]. In one form of MDS, refractory anemia with ring sideroblasts, IO was found in the mitochondria of erythroid precursors, probably due to a mutated SF3B1, the splicing factor 3b subunit [25].

**Hematopoietic stem cell transplantation**
Iron overload may be associated with and influence the outcome of transplantation of hematopoietic stem cells derived from the bone marrow or the peripheral blood. The IO can be due to the primary disease, such as thalassemia or MDS, as discussed above, or develop following blood transfusions before and during the conditioning phase or following the transplant [26].

**The causes of iron accumulation in hematological disorders**
In both the hereditary or acquired hemolytic anemias, the main causes of iron accumulation are RBC transfusions and increased iron uptake (ineffective erythropoiesis) (figure 1).

**Blood transfusions**
Under normal circumstances about 3 mg of iron circulates in the blood, with the transport of 20–25 mg iron per day between tissues and organs. Gastrointestinal absorption of dietary iron is 1–2 mg per day, with an equivalent amount lost by the turnover of GI epithelial cells. The body has no other mechanism for disposing of excess iron [27]. In RBC transfusion-dependent anemias, IO may accumulate quickly. Every unit of transfused blood contains 200–250 mg of iron, and an RBC transfusion requirement of 2 units per month will result in over 20 g excess of body iron in 4 years [28].

Blood transfusions, when given at high frequency, are the main cause of IO (transfusional IO) in all types of chronic anemia discussed above, but also in other anemias such as pyruvate kinase-deficiency [29], Fanconi anemia, Diamond-Blackfan anemia [30] and some other hematological diseases [31].

**Iron absorption**
Iron homeostasis is regulated by the 25 amino acid peptide – hepcidin, which is mainly produced in the liver and secreted into the circulation. It binds to ferroportin, an iron exporter on the surface of absorptive enterocytes, macrophages, hepatocytes,
and placenta cells. This binding induces ferroportin to be internalized and degraded, decreasing, consequently, the export of iron from these cells [32].

Hepcidin level depends mainly on its rate of production, which is modulated mainly by the iron status. The iron status reflects the balance of iron uptake, utilization, storage and mobilization. Normally, iron loading increases hepcidin expression resulting in reduced intestinal iron absorption. Inflammation, through production of pro-inflammatory cytokines such as interleukin-6, has a smaller effect [35]. This part of iron regulation is covered in other chapters of this publication. Under certain hematological conditions, iron absorption is high in spite of IO. Although in some patients with idiopathic sideroblastic anemia IO is due to a mutation in the HFE gene, which is involved in the classical hereditary hemochromatosis [33], in most cases it is due to low hepcidin levels [34]. This could be the result of several causes that are common in hemolytic anemias.

**Oxidative stress**

Due to inactivation of transcriptional factors, including CCAAT/enhancer-binding protein a (C/EBPa) and signal transducer and activator of transcription 3 (STAT3) [36], hypoxia-inducible factors [37], as well as by induced histone deacetylase activation [38].

**Increased utilization**

Patients with hyperplastic erythroid marrows absorb increased amounts of iron to the point where they may have clinical IO. Examples include the hereditary sideroblastic anemias, severe α- and β-thalassemia, and MDS variants such as refractory anemia with ringed sideroblasts (RARS). The low levels of hepcidin in these conditions could be explained by the enhanced erythropoietic activity (chronic stress erythropoiesis), which increases the demand for iron required for Hb production. In a mouse model of human β-thalassemia, hepcidin mRNA levels were lower in the livers of homozygous mice than in heterozygous mice and both had lower levels when compared to normal mice [39]. Conditions of hemolysis, bleeding, hypoxia, and administration of erythropoietin were found to decrease the expression of hepcidin in mice livers [40]. Our findings [41] support the concept that hepcidin levels in heavily transfused MDS patients represent a balance between the stimulating effect of IO and the inhibitory effects of stress erythropoiesis and oxidative stress. Although, in general, there

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**Figure 1**

**Iron overload and oxidative stress in hemolytic anemia**

Hemolytic anemias are associated with chronic anemia due to intra- and extra-vascular hemolysis as well as enhanced, but abortive, RBC production (ineffective erythropoiesis). The high demand for iron for the latter decreases hepcidin production and increases iron uptake, and iron overload. The patients are often treated by RBC transfusions, causing iron overload that increases (not shown) hepcidin production and decreases iron uptake. The net effect of RBC transfusions and hepcidin-regulated iron uptake determine the extent of iron overload in the patients.

**Acronyms:** RBC: red blood cells; ROS: reactive oxygen species; SCD: sickle cell disease; G6PD: glucose-6-phosphate dehydrogenase; CDA: congenital dyserythropoietic anemia; PNH: paroxysmal nocturnal hemoglobinuria; MDS: myelodysplastic syndrome; MPD: myeloproliferative diseases.
In erythroid cells, it is mainly incorporated into heme to form the protein hemoglobin, but a small fraction is taken up by non-transferrin pathways [49]. Iron gets into cells through their surface transferrin receptors (TfR1), the main erythroid cell regulator. This attempt is, however, abortive due to increased apoptosis and abortive maturation of the erythroid progenitors and precursors.

The mechanisms linking erythropoiesis to hepcidin production are: (A) The growth differentiation factor 15 (GDF-15) — its level in serum is correlated with the extent of overall erythroid maturation. It is increased approximately 100-fold in patients with severe thalassemia. It was demonstrated to suppress hepcidin mRNA expression in hepatocyte culture [43] (B) The soluble transferrin receptor — In various forms of erythropoietic dysfunction, including ineffective erythropoiesis, hepcidin deficiency was only found when high levels of GDF-15 were associated with high levels of the soluble transferrin receptor [44]. (C) Erythroferrone — In animal studies, bleeding or administration of erythropoietin led to the release of this factor made by erythroblasts that act directly on hepatocytes to suppress hepcidin production [45].

Iron overload mediated oxidative stress in hematological disorders

The deleterious effects of excess iron are mediated by oxidative stress. The cellular oxidative status is regulated by the equilibrium between oxidants, such as the reactive oxygen species (ROS) that are produced mainly as byproducts of the cellular respiration, and anti-oxidants, such as reduced glutathione. Balanced redox state is crucial for normal physiology; ROS serve as important regulators in many signal transmission pathways, including in the proliferation and differentiation of hematopoietic cells. When this balance fails, such as in many pathological processes, oxidative stress occurs. The excess ROS bind to various cell components such as the DNA, proteins and membrane lipids, leading to cytotoxicity. The interaction of ROS with various cell components depends on their site of generation and their life-span [1].

Most of the iron in the body is bound to molecules that render it redox-inactive. In the plasma, it circulates bound to transferrin [46]. When transferrin saturation is > 80%, a non-transferrin-bound iron (NTBI) appears in the plasma [47], including a fraction that is redox-active (labile plasma iron, LPI) [48]. Most of the iron gets into cells through their surface transferrin receptors (TfR1), but a small fraction is taken up by non-transferrin pathways [49]. In erythroid cells, it is mainly incorporated into heme to form the Hb molecule, or is stored in ferritin [50]. A small fraction of the incoming iron remains free or loosely bound to other compounds as the labile iron pool (LIP) [51] which was suggested as an intermediate or transitory pool between extracellular iron and intracellular firmly-bound iron [52]. The LIP participates in the Fenton, Haber-Weiss reactions and catalyzes the production of ROS. This sequence of events links IO to oxidative stress. Although the chronic anemias described above differ in their basic etiology, a large volume of evidence suggests that many of their symptoms are mediated by oxidative damage to their RBC as well as to their platelets and leukocytes [1]. A typical example is thalassemia: In β-thalassemia, excess α-globin chains form unstable tetramers that dissociate into monomers and then are oxidized to hemichromes which precipitate intracellularly [53]. Following the release of heme and iron, there is deposition of the protein moiety on the plasma membrane. The outcome of this chain of events is enhanced formation of ROS, catalyzed by free iron, with a variety of deleterious effects on the membrane lipids and proteins, including oxidation of the membrane protein band 4.1 and a decrease in spectrin/band 3 ratio [54]. In α-thalassemia, the γ- and β-globins, which are produced in excess, do not precipitate right away, but form the soluble tetramers αβ (Hb Bart’s) in the fetus and, later, the β4 (HbH), that are less stable than HbA and have an increased susceptibility towards oxidation and hemichrome formation [55].

The effects of RBC on oxidative stress are multifaceted. In an in vitro study, we have shown that incubation of various cells with normal RBC reduced their oxidative status, suggesting that RBC have an antioxidant effect (Dana and Fibach, in preparation). This property is reduced in thalassemic RBC compared to normal RBCs. It was decreased when normal RBC were treated with iron or oxidants, while thalassemic RBC regained this property when treated with iron chelators or anti-oxidants. These results suggest that RBC can function as antioxidants and that thalassemic RBC lose this property due to IO. In this regard, it is interesting to study the effect of RBC transfusions in patients with oxidative stress. On one hand, transfusions may increase the oxidative stress by causing IO, but on the other hand, the infused normal RBC may function as antioxidants. Another study suggested that the antioxidant activity of RBC is partially due to their ability to bind antioxidants such as the nutrition-derived polyphenols [56]. In contrast to their ameliorating effect, RBC undergoing intravascular hemolysis may release iron-containing compounds (Hb or heme), and thus add to the iron load and further aggravate the hemolysis or cause damage irrespectively.

Measurements of free iron and oxidative stress in blood cells:
Methods for quantification of iron in the serum and some organs for determination of IO have been presented in earlier chapters of this publication. In short, the most common clinical laboratory tests are measuring iron concentration, total iron binding capacity, transferrin saturation and ferritin in the serum [57]. The transferrin saturation is calculated from the serum iron concentration divided by the total iron binding capacity. Normal transferrin saturation range is 20–50%, the cutoff point is 60%.
for males and 50% for females. If the transferrin saturation is elevated, a serum ferritin assay should be performed. The former is, however, a more sensitive and specific test than the serum ferritin, which is an acute phase reactant and can be elevated due to a variety of reasons such as inflammation or malignancy. Iron overload is considered when serum ferritin exceeds 500 ng/mL, but it can reach up to > 10000 ng/mL. The non-bound iron forms, NTBI and LPI, are very specific for IO and have promising value for monitoring of the clinical status and response to chelation therapy [58]. However, the lack of standardization and limited experience necessitates further evaluation. Hepcidin measurement in the serum and the urine have been performed using mass spectrometry; this may be a feasible marker in the future [59].

Organ IO in hematological diseases could be evaluated based on liver iron content (LIC) following biopsy. This approach has been the "gold standard" for iron balance studies, but the technique is invasive, expensive, and subject to variability. It is now well established that some patients on long-term chelation therapy with high LIC have little or no cardiac IO, and vice versa [60]. Thus, LIC and its surrogate marker serum ferritin may not reflect myocardial iron status. Direct myocardial iron measurements should be regularly undertaken of patients with β-thalassemia and other forms of transfusional IO at risk of myocardial iron loading. Noninvasive quantification of organ iron deposition has been accomplished by measuring the magnetic resonance imaging (MRI) parameter T2*. T2* is a measure of magnetic relaxation, which is shortened when particulate hemosiderin storage iron disturbs the magnetic microenvironment, and it is easier to measure in the heart than T2 [63]. The T2* technique has been calibrated in animals [64] and humans [65]. As myocardial T2* falls, there is increasing risk of left ventricular dysfunction [66] and an increased likelihood of cardiac events [67]. T2* reports initially raised concerns about its usefulness as a good surrogate for cardiac IO [68] for technical reasons as well as the paradox that LIC correlated poorly with cardiac T2*. These points have largely been settled. In an IO gerbil model, T2* is indeed inversely related to myocardial iron measured directly [64]. The poor correlation of T2* with hepatic iron is now understood on a kinetic basis. The liver can be readily unloaded by aggressive chelation much more rapidly than can the heart, giving rise to patients with low T2* values of the heart while the liver has been unloaded to "safe" values [66]. Eventually, the T2* MR has been proven to be of good reproducibility [69] and was associated with reduced cardiac mortality [70].

Oxidative stress parameters can be measured by a variety of biochemical and physical tests [71], but are not routinely measured in the clinical laboratory. The most used assay for clinical purposes is the measurement in the serum of malondialdehyde (MDA) - a by-product of lipid peroxidation [72,73]. We have described a unique methodology to study IO and its consequential oxidative stress in blood cells by flow cytometry. Clinical flow cytometry is used mainly for characterization of hematological disorder such as malignancies (leukemia, lymphoma and multiple myeloma) and other conditions associated with abnormal blood cells (e.g., PNH). The expressions of various antigens are quantified simultaneously by staining blood or bone marrow samples with multiple fluorescent antibodies. Sub-populations of the examined sample are then characterized by their cellular fluorescence. Using appropriate fluorescence probes, we have adapted this methodology for quantification of cellular free iron and oxidative stress parameters [74,75]. Reactive oxygen species can be measured by staining cells with the non-polar compound, 2'-7'-dichlorofluorescin diacetate. It readily diffuses across the membranes and becomes deacetylated into esterases into a polar derivative that is trapped inside the cells. When it is oxidized by ROS (mainly peroxides), a green fluorescent product — dichlorofluorescin is produced. ROS can also be measured by dihydrorhodamine 123, which freely enters into cells and, after oxidation by ROS, emits a bright red fluorescence [76].

Reduced glutathione (GSH), the main cellular antioxidant, can be measured using mercury orange [76,77], which forms fluorescent adducts with GSH via the sulphydryl group, producing an S-glutathionyl derivative that emits red-orange fluorescence. The probe reacts more rapidly with non-protein thiols, such as GSH, compared with thiol-containing proteins, allowing specificity under controlled staining conditions. Other parameters measured by flow cytometry are membrane lipid peroxidation — by staining with N-(fluorescein-5-thiocarbamoyl)-1,2-dihexadecanoyl-sn-glycero-3-phosphoethanolamine [74], and externalization of phosphatidylserine, a marker of membrane damage, by fluorochrome-conjugated annexin-V [78]. Label iron (LIP) can be measured following staining with calcein AM, as previously described [75].

Using this methodology, we have shown high levels of LIP and oxidative stress parameters in blood cells of patients with hereditary and acquired hemolytic anemia [1]. These studies demonstrated that assays for these parameters are of significant importance for the diagnosis as well as for evaluating the efficacy of treatment, especially with iron chelators. While changes in other analytes could be non-specific or slow to develop (e.g., serum ferritin), changes in LIP and oxidation markers in response to treatment are fast [79]. Moreover, the assays can be simultaneously applied to RBC and various other blood cells, and thus detect defects in polymorphonuclear neutrophils and platelets, which may result in recurrent infections and thromboembolic complications, respectively.

**Treatment of iron overload**

Since the body has no efficient mechanism to get rid of excess iron, increased iron intake (due to absorption and/or RBC transfusions) results in its accumulation in cells and damage to major organs.
Iron overload in hematological disorders

Phlebotomy
The easiest way to remove excess iron is by bleeding (phlebotomy). However, it can be used only when the Hb levels are within normal range. Therefore, this mode of treatment is limited; the most common disease where phlebotomy is the major mode of treatment is Hereditary Hemochromatosis. Another group of patients is those after hematopoietic stem cell transplantation who had IO prior to transplantation due to multiple transfusions.

Iron chelation
Most of the other patients with IO are anemic (Hb < 10 g/dl), and therefore, particularly those who are transfusion-dependent, will require iron chelation therapy in order to achieve a transferrin saturation of < 50% and serum ferritin < 500 ng/mL. Currently, there are three iron chelators in clinical use.

Deferoxamine
The first drug used for iron chelation therapy. It is given by a slow overnight subcutaneous infusion through a portable pump for 5-7 nights/wk or via 24-h IV infusion. Doses are 1 to 2 g in adults and 20 to 40 mg/kg in children. Although its side effects are minimal, the severe disadvantage of Deferoxamine is its mode of administration – either parenterally or through a portable infusion pump on a daily basis. This therapy is complex and requires time commitment and compliance from the patients, resulting in a high rate of nonadherence. Adverse effects include hypotension, GI disturbances, and anaphylaxis (acute) and vision and hearing loss (chronic).

Deferasirox
An effective oral chelating agent that is increasingly used as an alternative to deferoxamine. Deferasirox reduces iron levels and prevents or delays the onset of complications of IO. The initial dose is 20 mg/kg per OS once/day. Patients are monitored monthly with dose increases of up to 30 mg/kg once/day. Treatment can be interrupted when serum ferritin is < 500 ng/mL. Adverse effects (which occur in about 10% of patients) can include nausea, abdominal pain, diarrhea and rash. Liver and kidney functions may become abnormal and should be tested monthly or more frequently for high-risk patients). Recently, film-coated tablets have been introduced. This formulation provides better patients’ adherence since it can be swallowed with a light meal, without the need to disperse it into a suspension or to take it with food.

Deferiprone
An oral iron chelator indicated for the treatment of patients with transfusional IO due to thalassemia syndromes as an alternative and/or when chelation therapy with deferasirox or deferoxamine is inadequate. Deferiprone was found to be very effective in removing excess iron from the organs and mainly from the heart. The initial dose is 25 mg/kg and maximum dose is 33 mg/kg per OS. Absolute neutrophil counts should be obtained weekly to look for neutropenia (which sometimes precedes agranulocytosis). Serum ferritin is measured every 2 to 3 months, and treatment is interrupted when levels are consistently < 500 ng/mL. A liquid formulation of deferiprone has been recently introduced [81].

A combination of chelators was demonstrated to be very effective. Based on the “shuttle mechanism”, iron is mobilized by deferiprone from tissues into the circulation where deferoxamine binds and excretes it in the urine.

Treatment with these chelators has been demonstrated to significantly reduce the morbidity and mortality of patients with IO by preventing or reducing the damage to key organs such as the heart, liver, and endocrine glands.

Additional aspects of iron chelation
As discussed above, various pathological aspects of IO are mediated by oxidative stress. By removing intra- and extracellular iron species that generate ROS, iron chelators act as antioxidants [2]. We have demonstrated the antioxidant effect of treatment with Deferasirox [79] and Deferiprone (Merkel et al. in preparation) in multi-transfused patients with MDS where amelioration of oxidative stress parameters was achieved after three months. In addition, attempts have been made to complement the chelation treatment with antioxidants [82]. Some compounds demonstrated dual effect as both iron chelators and antioxidants. For example, various antioxidative polyphenols [56] and antioxidant-containing nutritional additives (e.g., curcumin [83] and Fermented Papaya Preparation [84]), have been reported to have iron chelating properties as well. Interestingly, administration of exogenous iron-free (apo)-transferrin was demonstrated to decrease symptoms of IO in β-thalassemic mice [85], probably by binding NTBI.

In addition to reducing iron- and oxidative stress-induced toxicity to various organs, chelators may have additional beneficial influence in hematological disorders [4]: Acute leukemia can appear as either primary or as secondary malignancy. The latter may be the outcome of chemotherapy and radiotherapy, or develop consequently to chronic leukemia (e.g., CML, CLL), or MPD (e.g., polycythemia vera) or MDS. The acute phase of leukemia is characterized by an accelerated proliferation and defective differentiation of hematopoietic precursors [86]. Both abnormalities have been demonstrated to be corrected by iron chelation [87,88]. Another compound, Eltrombopag, a small, non-peptide thrombopoietin receptor agonist, activates the JAK-STAT signaling pathway, thereby increasing the proliferation and differentiation of megakaryocytes, and platelet production. In addition, it was reported to inhibit leukemia cell growth and to induce cell differentiation [89], and thus may have a beneficial impact on MDS patients [90] suffering from IO, thrombocytopenia and likelihood to transform into acute leukemia.

Disclosure of interest: the authors declare that they have no competing interest.
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