Too much iron: A masked foe for leukemias

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ABSTRACT

The role of iron in non-erythroid hematopoietic lineages and its implication in hemato-oncogenesis are still debated. Iron exerts an important role on hematopoietic stem cell transformation and on mature white blood cell differentiation. Iron acts experimentally as an oncogenic cofactor but its exact role in the transformation of the myelodysplastic syndrome into leukemia continues to be discussed. Body iron overload frequently develops mainly as the result of multiple erythrocyte transfusions in patients with leukemia or myelodysplastic syndrome, and, in the latter, as a result of increased ineffective erythropoiesis. Iron overload, especially through the deleterious effects of reactive oxygen species, leads to organ damage that likely impacts the global outcome of patients, especially after hematopoietic stem cell transplantation (HSCT). In these pathological settings (before and after HSCT), oral iron chelation should be considered whenever body iron overload has been firmly established, ideally by magnetic resonance imaging.

KEYWORDS

Iron; leukemia; myelodysplastic syndrome; hematopoietic stem cell transplantation; oral chelation; phlebotomy.

ABBREVIATIONS

ALL: acute lymphoblastic leukemia

AML: acute myeloid leukemia

DFX: deferasirox

HSC: hematopoietic stem cell

HSCT: hematopoietic stem cell transplantation

LIC: liver iron content

LPI: labile plasma iron

LPS: lipopolysaccharide

MDS: myelodysplastic syndromes

MDS-RS: myelodysplastic syndrome with ring sideroblasts

MSC: mesenchymal stromal cell

MRI: magnetic resonance imaging

NTBI: non transferrin-bound iron

ROS: reactive oxygen species

INTRODUCTION

The crosstalk between iron and hematology is illustrated by the well-known relationship between iron and erythrocytes where iron is required in order to sustain erythrocyte production within the bone marrow. Iron is abundantly found in the blood within these erythrocytes, and released during erythrocyte degradation mainly within the spleen (1). The goal of the present review, beyond examining the role of iron in the erythroid lineage, is to focus on the relationship between iron and hematopoietic cells, with a special emphasis on iron overload and leukemias. Systemic iron overload frequently develops in leukemias, mainly due to transfusion therapy (2-4). Another mechanism, involved in myelodysplastic syndromes (MDS) in particular (hematologic malignancies characterized by a risk of progression to acute myeloid leukemia (AML)), is ineffective erythropoiesis (5). Ineffective erythropoiesis, notably through increased bone marrow production of the hormone erythroferrone (6), reduces hepcidin expression (7), leading to increased digestive absorption of iron and increased splenic release of the iron originating from erythrophagocytosis (8). After a reminder on the physiological role of iron in bone marrow stem cells and mature white blood cells, the following pathological aspects will be considered: the body iron load status in leukemia patients both at diagnosis and during medical care, the negative consequences of iron excess on patient outcome, especially after hematopoietic stem cell transplantation (HSCT), and the clinical interest of removing excessive iron in leukemia patients.

1. IRON: HOMEOSTASIS AND FUNCTIONAL LINKS BETWEEN IRON AND BONE MARROW

1.1. General iron homeostasis

Iron is critical for life due to its major roles in oxygen transport, key enzymatic reactions (such as DNA synthesis or detoxification) and the growth of microorganisms. In vertebrates, iron is abundantly present within red blood cells and in a tiny but functionally essential amount in

the plasma; this plasma iron ensures the delivery of iron to the bone marrow primarily for erythrocyte synthesis and to all living cells (9-11) (Fig. 1). Iron is stored in parenchymal cells (essentially hepatocytes where it is sequestered within ferritin molecules) and in macrophages (mainly splenic macrophages). The only physiological source of iron for the body is alimentary, explaining the risk of iron deficiency when this input is limited. In the case of excessive iron input, either by increased intestinal absorption (such as in hemochromatosis) or by excessive parenteral entry (due to multiple transfusions or excessive iron supplementation), there is no effective possibility for the human body to adapt its excretory pathways, which explains the risk of iron overload. This dual vulnerability to iron deprivation and to iron excess explains that iron homeostasis is very finely regulated, both at the systemic and cellular levels. At the systemic level, the key role belongs to hepcidin, the major hormone regulating iron metabolism. Hepcidin is a small peptide of 25 amino acids, essentially produced by hepatocytes, that decreases the plasma iron concentration. The decreasing effect exerted by hepcidin on the plasma iron level is mediated by the degradation of ferroportin, which is the only known protein to export cellular iron into the plasma. This hepcidin-ferroportin duo acts at two main sites, duodenal enterocytes and the spleen. At the digestive level, through the combined action of ferroportin export and hephaestin oxidation, iron is delivered to plasma transferrin which principally targets the bone marrow in order to produce new red blood cells (see Table 1 for names and symbols of the genes). In the spleen, iron originating from erythrophagocytosis is exported into the plasma through ferroportin, oxidized by ceruloplasmin, bound to transferrin and recycled toward the bone marrow. Many factors can regulate hepcidin expression. Body iron load status is a major regulator: a decrease in iron levels (either plasma transferrin saturation level and/or intrahepatocyte iron load level) leads to lower hepcidin production in order to counteract iron deficiency. The reverse phenomenon occurs in the case of iron overload. Inflammation is also an important regulatory factor, in which the IL6-STAT3 pathway is involved to increase hepcidin production.

The erythroferrone hormone, mainly produced by the bone marrow, is another important regulatory factor (12, 13). Its increased production in the case of dyserythropoiesis leads to the downregulation of hepcidin expression. This decrease in hepcidin production may override the expected increased hepcidin expression in the presence of coexisting iron excess. At the cellular level, iron homeostasis is mainly ensured at a post-transcriptional level by the iron regulatory proteins (IRP). Schematically, in a context of cellular iron deficiency, IRP1 and IRP2 bind to iron regulatory elements (IRE) located in the 5' or 3' untranslated transcribed region (UTR) of the mRNA involved in the entry (TFR1), storage (ferritin) and export of iron (ferroportin). This leads, through different mechanisms (inhibition of translation or stabilization of transcripts), to increased cellular iron entry and decreased cellular iron storage and egress. The reverse effects occur in the case of cellular iron overload, where IRP1 and IRP2 lose their capacity to bind to IRE. Any acquired and/or genetic abnormality in these regulatory processes may lead to iron-related disorders.

Altogether, many of the players involved in iron metabolism are now described at the molecular level. They are transporters, receptors, oxido-reductases, sensors, chelators, regulatory pathway players and hormones. Their interdependency and fine-tuning explain the functional relationship between diverse tissues and iron.

1.2. The functional relationship between iron and bone marrow

There is a very close relationship between iron and bone marrow that can be considered through two reciprocal perspectives. On the one hand, there is an iron flux toward the bone marrow that is physiologically required to sustain crucial hematopoietic functions, and which, when excessive, can exert deleterious effects on hematopoiesis (14-16). On the other hand, the bone marrow not only ensures the delivery into the blood of the different hematological cells involved in iron

metabolism (the erythrocytes in first line), but also plays a key role in the systemic distribution of iron.

1.2.1. From iron to the bone marrow: iron is used by all hematopoietic lineages

1.2.1.1. Iron and bone marrow stem cells: hematopoietic and mesenchymal stromal cells (HSC and MSC)

Iron, through its propensity for producing reactive oxygen species (ROS), exerts a determining role on the fate of HCS (14, 15) and its impact depends on the dose of iron. Low ROS levels are required for HSC renewal. ROS levels also fine-tune HSC differentiation: very low levels can hamper HSC differentiation, whereas increasing ROS levels favor their differentiation. On the contrary, very high ROS levels promote stem cell exhaustion and death by causing cytotoxicity through the binding of ROS to lipid membranes, proteins and DNA, promoting deleterious oxidation of these macromolecules. Excessive iron impairs hematopoiesis by inducing apoptosis and cell cycle arrest (17), and decreases the ratio and clonogenic function of hematopoietic stem and progenitor stem cells (18). Iron also plays a role in other cells in the hematopoietic niche. Iron increases the proliferation of human MSCs and accelerates their entry into S-phase (19). In contrast, excess iron negatively impacts the hematopoietic microenvironment, as shown by the delayed hematopoietic reconstitution observed in mice overloaded by iron-dextran and transplanted with bone marrow from untreated mice (20). Moreover, in MDS patients, iron overload has been shown to promote mitochondrial fragmentation of mesenchymal stromal cells (21).

1.2.1.2. Iron in white cell lineages

A short history… In 1952, CB Laurell wrote that "nobody has investigated whether the white blood cells have any function in iron transportation or hemoglobin breakdown, in spite of the fact that leucocytes have a relatively high iron content" in a seminal review entitled "Plasma iron and iron transport in the organism" (22).Twenty-two years later, Summers and Jacobs (23, 24) showed that monocyte ferritin content was approximately seven times higher than that in lymphocytes or polymorphs in normal subjects. They also reported that the ferritin content increased during anemia of chronic disease in lymphocytes, monocytes and polymorphs. Iron uptake by lymphocytes was also greatly increased during iron deficiency anemia. At the same time, Worwood *et al.* reported that leukemia cells had a markedly increased ferritin content (25). We will now consider the present state of knowledge about iron content, function and impact in white blood cells.

Iron and monocytes-macrophages. Monocytes are known to correspond to circulating macrophages. Iron is required for the differentiation of peripheral blood monocyte precursors into functional macrophages; iron deprivation by desferrioxamine generates macrophages unable to develop a mature phenotype, with an impaired capacity for phagocytosis (23). It is also well known that iron sequestration occurs within macrophages during inflammation. Importantly, interleukin 6 (IL-6), which is produced by macrophages in response to pathogen-associated molecular patterns, activates the STAT3 signaling pathway leading to hyperhepcidinemia, and therefore to the degradation of ferroportin, and eventually to hyposideremia. It has also been shown that inflammation can cause hyposideremia through a hepcidin-independent mechanism involving the transcriptional downregulation of ferroportin (26). Depending on their microenvironment and cooperation with lymphocytes, monocytes polarize into active macrophage populations that are subdivided into activated M1 macrophages exerting proinflammatory and antitumor activities, and into activated M2 macrophages that are immunosuppressive and promote tumor activity. Importantly, iron modulates this macrophage polarization, increasing the M2 phenotype and decreasing the M1 proinflammatory lipopolysaccharide (LPS)-induced response (27). In a mouse model of hemochromatosis (a genetic iron overload disease), attenuated inflammatory responses

to *Salmonella* infection or LPS were observed compared to non-hemochromatosis control mice. This study demonstrates a novel role of iron in the regulation of macrophage IL-6 cytokine mRNA translation (28). This result is coherent with a previous report that showed that TNFα concentrations were decreased in the supernatants of monocytes from hemochromatosis patients (29). Youssef *et al.* (30) reported that, following increased erythrophagocytosis, splenic macrophages undergo ferroptosis and are replaced by circulating monocytes and local cell division. Haschka *et al.* (31) found that classical ($CD14^+$, $CD16^-$) and intermediate ($CD14^+$, $CD16^+$) subsets of human monocytes are involved in clearing non-transferrin-bound iron (NTBI) and damaged red blood cells. Therefore monocytes may play a role in limiting iron toxicity in iron overload conditions.

Iron and dendritic cells. Kramer *et al.* showed that differentiation from human peripheral blood monocyte precursors into functional dendritic cells requires iron and is dependent on the expression of the cyclin-dependent kinase inhibitor p21 (32). Iron depletion by the chelator desferrioxamine produced undifferentiated dendritic cells unable to stimulate naive allogeneic T lymphocytes. Olakanmi *et al.* showed that lung myeloid dendritic cells acquire iron from various types of extracellular sources (iron-citrate, iron-transferrin and iron-lactoferrin) (33).

Iron and neutrophils. The positive role of iron on granulopoiesis is suggested by studies in anemic Belgrade (b/b) rats. Indeed, an increased proliferation of granulocytic cells is observed in iron-treated b/b rats (34). Importantly, neutrophils impact iron metabolism during inflammation by secreting lactoferrin (35, 36). Lactoferrin is an iron-binding multifunctional glycoprotein secreted by neutrophils and exocrine glands. During inflammation or infection, the plasma lactoferrin concentration increases through the recruitment of neutrophils (37). The antibacterial and antibiofilm activity of lactoferrin is dependent, although not exclusively, on its iron-binding affinity. However, no evidence has been brought forward regarding a significant impact of lactoferrin on human granulopoiesis (38).

Iron and eosinophils. To our knowledge, the sole interference of iron with the eosinophil lineage involves the role of iron-loaded lactoferrin. Bovine lactoferrin regulates the pathway implicating the CD11b and CD49d integrins and the MIP-1α and MCP-1 chemokines in granulocyte-macrophage colony-stimulating factor (GM-CSF)-treated human eosinophils (39).

Iron and lymphocytes. Transferrin receptor 1 (TFR1)-mediated iron uptake is essential for lymphocyte proliferation (40). Iron also plays a key role in the differentiation of T lymphocytes. The absence of TFR1 in genetically modified mice leads to a total arrest of T lymphopoiesis at a very early stage of maturation, and the function and impact of iron on B lymphocytes is less pronounced (41). A significant increase in lymphocyte number is observed in iron overload situations such as human *HFE*-related hemochromatosis (42). The number of CD8+ T lymphocytes negatively correlates with the severity of iron overload in *HFE*-related hemochromatosis (43). Lymphocytes can also internalize iron through pathways involving non-transferrin-bound iron (NTBI) (44, 45), supporting the view that T lymphocytes may play a protective role against the consequences of iron excess. This result is in accordance with the proposal that T lymphocytes act as a first line protective barrier against the deleterious effects of iron excess (46). The cellular localization of iron was not addressed in this study but the authors suggest that it may be stored within ferritin, in agreement with the demonstration that T lymphocytes are able to synthetize H-ferritin (47).

In summary, all hematopoietic lineages use iron for proliferation and/or differentiation functions, including the white blood cell lineages. The physiological flux of iron toward the bone marrow exerts proliferative and differentiating effects on HSCs and MSCs. An excess of iron within the bone marrow impairs these processes, mainly through an excessive production of ROS. Regarding mature white blood cells, iron is required for the maturation and phagocytotic activity of peripheral monocytes, the differentiation of monocytes into functional dendritic cells, the proliferation of granulocytic cells, and T lymphopoiesis.

1.2.2. From the bone marrow to iron: bone marrow is a prominent player in systemic iron metabolism

Bone marrow, erythrocyte production, and spleen erythrocyte degradation participate in the systemic distribution of iron. Once produced inside the bone marrow, erythrocytes are released into the plasma in order to help oxygenate the body. Circulating red blood cells contain half the total quantity of whole-body iron, *i.e.* approximately 1.5 to 2 g of iron. After 120 days, senescent erythrocytes are degraded within macrophages that release iron from hemoglobin. Iron then re-enters the iron cycle without being excreted.

Bone marrow also plays a critical role in systemic iron regulation by modulating the expression of hepcidin (Fig. 2). The main bone marrow factors that may ultimately regulate hepcidin are GDF15 (growth and differentiation factor 15) (48), TWSG1 (twisted gastrulation BMP signaling modulator 1) (49), and the hormone erythroferrone (encoded by the gene *ERFE*) (6, 7, 12). During dyserythropoiesis, as seen for instance in thalassemia major or intermedia (50-52) or in MDS (5, 53), those factors secreted by the bone marrow may negatively impact hepcidin synthesis, leading to hepcidin deficiency and, in turn, to hypersideremia and subsequently body iron overload (54-56), even prior to any transfusion. Bair *et al.* (57) showed that allogeneic T-replete stem cell transplantation alters iron homeostasis in non-obese diabetic/severe combined immunodeficient (NOD/SCID) mice, by downregulating liver hepcidin synthesis ahead of upregulating duodenal ferroportin .

In summary, we have shown in this first section that iron is an essential player of bone marrow functions through its implication in proliferation/apoptosis and differentiation of all hematopoietic lineages. In return, the bone marrow impacts the systemic homeostasis of iron by the massive release of iron after erythrophagocytosis, the major source of circulating iron, and by modulating two crucial hormones of iron metabolism, hepcidin and erythroferrone. Having described the normal functional

relationships between iron and the bone marrow, we next explore pathological aspects of this relationship. In the following sections, we report the causative links between iron and leukemia and their clinical implications.

2. IRON STATUS IN LEUKEMIAS

2.1. The most suitable methods to assess body iron load in patients

Evaluating the total body iron load requires the use of reliable tools. In particular, it may not be sufficient to rely exclusively on plasma ferritin levels in light of the numerous non-iron-related factors susceptible to induce hyperferritinemia (58, 59). In leukemia patients, these factors include inflammation (60), the metabolic syndrome (61) and a high ferritin content within leukemic cells (25). It should also be noted that plasma ferritin levels not only depend on the amount of cellular iron deposition but also on the cellular iron distribution. For equivalent cellular iron concentrations, the corresponding plasma ferritin levels are higher for macrophagic (typically transfusional) iron than for parenchymal (hepatocyte) iron, as typically observed in iron excess related to ineffective erythropoiesis (62). The clinician must keep this difference in mind since it may impact the threshold ferritin values used for clinical decision-making when treating iron overload. Combining plasma ferritin and transferrin saturation levels is especially informative, widely available, and cost-effective for the clinician, although both measurements can be affected by inflammation and must be interpreted with caution in this setting. In addition, ferritin can be affected by liver damage, and transferrin saturation fluctuates diurnally and is also affected by cytolysis (63). However useful these iron-related blood parameters may be, the main message is that it is of utmost importance to rely on the direct visualization and quantification of the tissue iron content (especially in the liver and spleen) in order to rigorously assess the body iron load. Non-invasive approaches such as magnetic resonance

imaging (MRI) should be preferentially used (64-67). MRI to determine the liver iron concentration (LIC) has now largely replaced histological assessment by liver biopsy. However, MRI is not widely available nor uniformly done. Bone marrow iron evaluation using MRI, the interest of which has recently been reported for Gaucher disease (68), has not yet been fully evaluated in hematologic malignancies (69). As an illustration of the superiority of MRI evaluation as compared to serum ferritin for body iron load assessment, no hepatic iron overload was found in 13 out of 39 patients who had a serum ferritin level over 1000 ng/mL (70) (71). The bone marrow iron score could be an interesting indicator of secondary iron overload in acute myeloid leukemia patients (72). Dual-energy computed tomography (73) may be a promising technique for the precise assessment of intrahepatic iron distribution in transfusion-dependent patients with hematological malignancies (74).

In summary, the assessment of body iron stores is a two-step process. First, together with the hemoglobin level, the reticulocyte count, red blood cell morphology, serum ferritin and transferrin saturation must be checked. If both the ferritin and transferrin saturation parameters are normal, reflecting an absence of iron excess, no further investigations are required. If they are elevated, and after having checked for possible confounding factors, a direct evaluation of the tissue iron load is required. MRI has currently become the preferred approach given its non-invasive nature, provided it is available and affordable. Among the various methods, the signal intensity ratio method (64, 75) is quite promising as it enables the direct assessment of the hepatic and splenic iron load without requiring specific MRI equipment and provides free interpretation.

2.2. Iron status at diagnosis

It remains difficult to obtain precise data on the body iron status at the time of diagnosis since the iron load check-up is usually done during or after chemotherapy and often from the perspective of HSCT. However, Vag *et al*. (76) reported that LIC measured by MRI was close to normal in eight patients with acute leukemia for whom the determination was performed within 10 days after

therapy initiation. Moreover, the study by Moafi *et al.* (77), based on a histological evaluation of the bone marrow iron stores, reported that, when 30 acute lymphoblastic leukemia (ALL) patients at diagnosis were compared with 30 control subjects, the bone marrow iron score did not differ significantly whereas a significant elevation appeared in leukemia patients after one year of chemotherapy.

Altogether, those limited data suggest that the iron status at diagnosis of acute leukemia is not different from control subjects. However, larger cohorts and prospective studies are required to definitively conclude of this point.

2.3. Body iron load increases in leukemias, mainly due to iron input from blood transfusions

Body iron status has mostly been evaluated while leukemia was being treated. Altogether, the number of studies remains limited when referring to the direct evaluation of body iron stores using appropriate techniques (MRI or histology). Halonen *et al.* reported that 30 children with acute lymphoblastic leukemia (ALL) of whom 19 (63%) had moderate iron overload as assessed by the total iron score (78, 79). Vag *et al.* (76) showed that the mean LIC of 15 children (nine ALL, six AML (acute myeloblastic leukemia)) was significantly increased compared to non-leukemic children. The mean LIC was then correlated with the number of transfusions. Armand *et al.* (80) found that 85% of 48 patients with AML (n=29), ALL (n=11) or MDS (n=8) had a LIC value above the upper limit of normal and, in 42% of patients, significant iron excess corresponded to more than three times the upper limit of normal. Again, iron overload was correlated with the number of transfusions. In Trottier *et al.*'s cohort (4) consisting of 37 leukemia case, eight out of 10 ALL patients and all of the AML patients had an iron overload. A poor relationship was found between LIC and transfusion history. Maximova N *et al.* performed an MRI-based evaluation of multiorgan iron load in pediatric patients who subsequently underwent hematopoietic stem cell transplantation (HSCT) (69). Among these patients, 21 had ALL and eight had AML. Thirteen ALL and all eight AML patients had a significantly elevated

LIC (>2-3 times the upper limit of normal) after HSCT. LIC was not found to be a reliable indicator of total body iron stores as indicated by marked discrepancies between LIC data and the iron estimation in the spleen or bone.

On the whole, for the previously mentioned studies, 49% (91/185) of the ALL or AML patients had significant iron overload before HSCT. In these hematologic malignancies (2, 3), transfusions appeared to be the main cause of the iron burden.

3. IS IRON A PREDISPOSING FACTOR TO LEUKEMIA?

3.1. The potential cellular toxicity of iron

Physiologically, most iron in the body is incorporated within molecular moieties that make it redox-inactive. For instance, iron is bound to plasma transferrin that ensures its transport, and iron is stored within cells in ferritin macromolecules that act as sponges preventing “unbound” or “free” cytosolic iron from being toxic. At the cellular level, a large quantity of iron is incorporated into heme as part of the hemoglobin molecule in erythroid cells, or myoglobin in muscle cells.

However, this protective process can be overridden in iron excess. This holds true when the plasma transferrin saturation with iron is over 45%, with the appearance of non-transferrin-bound iron (NTBI), and especially when the transferrin saturation becomes higher than 75% with the appearance of labile plasma iron (LPI). NTBI is thought to be under the biochemical form of low-molecular weight complexes (such as citrate and acetate), whereas LPI represents the potentially toxic form of circulating iron, defined by its propensity to generate reactive oxygen species (ROS), capable of damaging the membranes of the cells, intracellular organelles and nuclei (17, 81, 82).

In summary, while iron is physiologically redox-inactive, iron excess, characterized by a plasma transferrin saturation with iron over 45%, is toxic for the cells.

3.2. Experimental data converge to indicate the promoting role of iron on leukemia development

The promoting role of iron on tumor development has been explored in numerous types of cancer (83) and the proliferative effect of iron is well documented (84, 85). Iron is also known to favor genetic instability (82). Focusing on leukemia models, the following data were reported. Inoculated L1210 cells (a mouse lymphocytic leukemia cell line) proliferated more in mice injected with iron than in non-iron-treated control animals (86). Further experimental data shed some light on the possible mechanistic impact of iron on tumorigenesis. Iron has been shown to reduce tumor suppressor p53 activity (87); however, two types of data do not fit with a promoting role of iron on tumorigenesis through decreasing p53: on the one hand, iron favors ferroptosis, whereas the mechanism by which p53 could sometimes favor tumorigenesis has been reported to be its suppressing effect on metabolic stress-induced ferroptosis (88); on the other hand, p53 has been shown to decrease the NTBI transporter ZIP14 (also known as SLC39A14) (89), leading to decreased NTBI entry into tumor cells and subsequently to decreased iron-related cell death. The precise role of iron on p53 regulation and its effect needs further clarification. The Eltrombopag effect further illustrates the promoting role of iron on leukemia cell growth. This compound is an oral small-molecule thrombopoietin receptor (TPO-R also known as MLP) agonist used for treating chronic immune thrombocytopenic purpura. However, independently of its TPO-R mediated effect, Eltrombopag is able to inhibit the growth of human and murine leukemia cell lines by inducing differentiation and by slowing cell division through blocking the cell cycle in the G1 phase. Interestingly, Eltrombopag decreased the iron content within leukemic cells in a dose-dependent manner. Preloading cells with iron also resulted in a rescue from the anti-proliferative and differentiation-inducing effects of Eltrombopag, suggesting that the antileukemic effect of Eltrombopag is mediated through intracellular iron content (90). Iron deprivation has been reported to induce monocyte differentiation of AML cells (91) and, more generally, cellular iron-binding may be an interesting approach to counteract cancer through the involvement of various signaling pathways (92). Finally, dihydorartemisinin has been reported to

induce acute myeloid leukemia cell death by inducing ferroptosis through the autophagy-dependent degradation of ferritin (93).

In summary, experimental preclinical data converge to indicate the promoting role of iron on leukemia development, and conversely, the role of iron chelation/deprivation to counteract cancer.

3.3. Is systemic iron overload in non-malignant hematological conditions a predisposition to leukemia?

3.3.1. Lessons from hemochromatosis, the archetype of systemic iron overload

Hemochromatosis is a genetic disorder characterized by diffuse body iron overload caused by increased intestinal iron absorption, which itself is related to hepcidin deficiency (10). *HFE* is by far the most frequently mutated gene found in hemochromatosis, with *C282Y HFE* mostly present in Caucasians. It is still being debated whether or not *HFE*-related genetic susceptibility to hemochromatosis, and therefore iron excess, could favor the development of leukemia. A causative link between the *C282Y HFE* mutation and ALL has not been clearly proven (94, 95) and the same holds true for an association with the haplotype HLA-A3, known to be closely associated with the *HFE* gene (95). However, in a US cohort of 161 childhood ALL cases, Kennedy *et al*. (96) reported that the risk of ALL was not only associated with *C282Y* and *H63D HFE* variants and *S142G TFR1* (transferrin receptor 1) variant, but also to some SNPs in other iron regulatory genes (*SLC11A2* and *TMPRSS6* genes). Although no data were given on the iron status of this cohort, these results strengthen the view that iron overload mediated by genetic variants could contribute to a risk of ALL. No *HFE* association has been reported with AML (97). It should be noted that only exceptional cases associating *HFE*-related hemochromatosis and ALL have been reported (98).

Altogether, a genetic correlation has been found between some variant genes involved in iron metabolism and ALL. The impact of the level of the iron burden is not clearly demonstrated as a cause of those ALL. Moreover, in our opinion, despite the absence of an established link between

hemochromatosis (iron overload) and leukemia, it is important in clinical practice to check *HFE* mutations and, if negative, *non-HFE* mutations, in every leukemic patient with marked iron overload exhibiting the characteristic profile of hepcidin deficiency (increased plasma transferrin saturation associated with parenchymal, especially hepatocyte, iron overload (10, 52)) so as not to miss a coincidentally associated hemochromatosis (10).

3.3.2. Lessons from sickle cell disease and thalassemia, archetypes of secondary iron overload

Sickle cell disease and thalassemia are inherited disorders treated with chronic blood transfusions resulting in diffuse body iron overload. Here again, very little data are available on the promoting role of iron overload in leukemia transformation in these diseases. In sickle cell disease, an over two-fold increase in the risk of leukemia, mostly AML, has been reported in California (99) although it is still unclear if iron overload was one of the risk factors in this study. In thalassemia, only exceptional cases of leukemia have been described, suggesting a coincidental occurrence of these conditions (100).

3.4. Is iron overload a factor favoring the transformation of myelodysplastic syndrome (MDS) into leukemia?

MDS represents a particularly valuable experimental model and clinical situation for studying the role of iron as a leukemia predisposing factor since MDS evolves towards AML transformation in approximately one third of cases (101) (102). MDS encompasses a heterogeneous group of acquired myeloid disorders leading to ineffective hematopoiesis with peripheral cytopenia(s) (103), among which anemia is the most frequent. Patients with MDS can develop iron overload through repeated blood transfusions and/or as a consequence of dyserythropoiesis.

3.4.1. Iron-related oxidative stress in MDS

It is now clear that MDS patients present increased oxidative stress that is further increased by iron overload (104, 105), even in transfusion-independent cases (105). It has been proposed (106) that iron deprivation by low-dose deferasirox can improve erythropoiesis in MDS. It should be noted, however, that iron overload inhibited the proliferation of erythroid progenitor cells in MDS whereas the myeloid compartment was not affected (107). All of the blood marrow cell types in 27 MDS patients exhibited high ROS and low glutathion levels compared to 12 controls, with some correlation with overall survival (108). Interestingly, Ghoti *et al*. found a correlation between ROS and serum ferritin levels in the erythrocytes and platelets of low-grade MDS patients (109). Increased oxidative DNA damage was found in MDS patients both on bone marrow cells (110) (however without correlation with serum ferritin) and on peripheral blood mononuclear cells (111), with a protective effect of oral iron chelation. Importantly, low-risk MDS patients have been reported to present the highest ROS levels (108). Using NHD13 transgenic mice, a murine model of MDS, Chung *et al*. showed that ROS may contribute to the progression of MDS to AML through ineffective DNA repair and increased mutation frequency (112). Given the close relationship between iron and ROS, it is possible that this progression was at least partly related to an iron effect (iron exposure, however, was not used in this experiment).

3.4.2. The impact of dyserythropoiesis and erythroblastic mitochondrial iron overload in MDS to AML transformation

Iron overload due to dyserythropoiesis is mainly found in MDS with ring sideroblasts (MDS-RS), a subgroup characterized by erythroblastic mitochondrial iron overload and a high frequency of somatic mutations in the spliceosome gene *SF3B1* (113). Mean hepcidin levels are heterogeneous across the different MDS subtypes, with the lowest levels in refractory anemia with ring sideroblasts (now classified as MDS-RS with single dysplasia), which present the highest plasma NTBI levels (114-116). Thus, MDS-RS patients have inappropriately low hepcidin levels, a typical feature of iron-

476 loading anemia (117). Most patients with MDS-RS with single dysplasia are stratified into the lower-
477 risk groups by the revised IPSS (118), pointing out that abnormal mitochondrial iron accumulation
478 may not be a strong factor favoring leukemic transformation.
479
480 3.4.3. The impact of transfusional systemic iron overload in MDS to AML transformation
481
482 It has been reported that chronic red blood cell transfusions induce an iron overload that impacts
483 the survival of MDS patients (119). In a retrospective study of 467 MDS patients, the leukemia-free
484 survival of transfusion-dependent patients was significantly inferior to that of patients not requiring
485 transfusions, raising the possibility that transfusional iron overload may increase the risk of AML
486 transformation to leukemia (120). However, in a recent Brazilian study, hepatic iron overload,
487 measured by MRI and found in two-thirds of transfused and non-transfused MDS patients, was
488 associated with a lower overall survival, but was not correlated with an increased risk of AML
489 transformation (121). These results should nevertheless be considered in the light of the small cohort
490 size and the heterogeneous clinical status of the patients.
491 Although clinical data converge to indicate that blood transfusion dependency and an increasing
492 number of transfusions favor shorter overall survival and increase leukemic transformation in MDS
493 patients, it remains difficult to ascertain that these deleterious effects are related to iron overload
494 itself (5). Limiting and confounding factors may include: i) the fact that transfusion dependency could
495 reflect the overall disease severity; ii) the impact of comorbidities that are frequent in these elderly
496 patients; iii) the almost exclusive use of plasma iron parameters (especially ferritin levels) to estimate
497 body iron excess, which does not consistently reflect the iron store (as discussed in 2.1); and iv) the
498 risk of selection bias related to the possible proposal of chelation therapy to patients with a better
499 prognosis. Evaluating the impact of iron depletion by chelation therapy represents a “reverse”
500 approach to understanding the effect of iron excess on MDS to AML transformation. In this regard,
501 the repeatedly-found beneficial effect of iron chelation therapy (106, 122-126) represents an

important argument in favor of iron overload being responsible for promoting AML. In particular, the TELESTO trial by Angelucci *et al*. (127), which is the sole prospective study randomizing deferasirox *vs*. placebo in low-risk MDS patients, reports a favorable effect of iron chelation on event-free survival, the rate of cardiac and hepatic events and the transformation to AML. However, it should be kept in mind that part of the beneficial effect of the iron chelator deferasirox may be due not only to iron chelation, but to well-documented associated hematopoietic effects (106, 125, 128-134). Finally, regarding the specific effect of iron chelation on AML transformation, the Spanish Iron2 study (126) identified a beneficial effect, although the German registry did not (123).

In summary, while systemic iron overload observed in hemochromatosis and sickle cell disease is not clearly shown as a predisposing condition to leukemia, data are clearer for MDS. Some experimental studies suggest that iron may be a factor facilitating MDS to AML transformation, and bioclinical data show that there is an increase in ROS production in MDS likely causing oxidative stress, including oxidative DNA damage. Clinical data are still lacking, however, to firmly establish the causal relationship between iron overload and leukemia transformation in MDS.

4. THERAPEUTIC ASPECTS RELATED TO IRON OVERLOAD IN LEUKEMIA

4.1. Leukemia treatment and body iron burden

4.1.1. The impact of chemotherapy on iron metabolism: the transient appearance of NTBI

Chemotherapy impacts iron metabolism through two mechanisms. The first one is that chemotherapy causes iron excess when red blood cell transfusions are needed to counteract chemotherapy-related anemia. A high amount of iron bound to hemoglobin is brought together

with massive erythrocyte supplementation. This iron, released from the erythrocytes during erythrophagocytosis, will not be extracted out of the body since no specific iron excretion exists, and will be stored within the organism. This corresponds to a net addition of iron. Another mechanism is that chemotherapy favors the appearance of plasma NTBI. Harrison P *et al*. were the first to report the presence of NTBI during chemotherapy, often concomitant with neutropenia (135). In 23 out of 25 patients, labile plasma iron (LPI) levels increased 48 hours after the start of conditioning pre-autologous HSCT, with a peak before cell infusion and a return to the normal range at engraftment (136, 137). Studying a cohort of 30 patients with acute leukemia (16 AML and 14 ALL), Belotti *et al*. (138) showed that this peak of NTBI was found for all subsequent high-dose chemotherapy courses. The appearance of NTBI during chemotherapy may be essentially due to the massive iron release from the degradation of hemoglobinized bone marrow cells leading to elevated transferrin saturation (139). The presence of NTBI can also be explained on the one hand by a decrease in NTBI uptake by the erythroid cells, given that such uptake has been demonstrated in rat erythroid cells (140). Furthermore, it could be related to hypohepcidinemia due to the absence of erythropoiesis. These different mechanisms can act simultaneously. Finally, high NTBI levels were associated with a higher risk of sepsis (138). It should be noted that susceptibility to infection may be, by itself, an important factor in terms of the survival of patients with leukemia undergoing chemotherapy.

As a whole, chemotherapy causes iron excess when red blood cell transfusions are provided and favors the appearance of plasma NTBI.

4.1.2. The prognostic impact of body iron burden on hematopoietic stem cell transplantation (HSCT)

Numerous studies have concluded, overall, that high serum ferritin levels prior to HSCT are a predictive factor of poor prognosis both in terms of morbidity and mortality. However, as previously

mentioned, hyperferritinemia clearly does not only reflect body iron overload, as this is notably an inflammation marker (see reviews by Moukalled *et al.* (101), Isidori *et al.* (15); Leitch *et al.* (141), Wang *et al.* (142), and Bertoli *et al.* (60)). This is why, in the present review, only studies using a direct assessment, by MRI or histological studies, of the tissue iron concentration will be considered.

A meta-analysis performed by Armand *et al.* (143) of four prospective studies (144) (4, 145, 146), involving 144 AML, 90 ALL and approximately 50 MDS patients, concluded that iron overload was not related to either overall survival or to non-relapse-mortality. However, one of the four studies (145) was not in agreement with the other three, and meta-analysis bias due to a sample size issue could not be totally excluded. Primarily, Wermke *et al.* (147) coordinated the first prospective, German multicenter observational study (the ALLIVE study) assessing the relevance of pretransplantation body iron overload measured by serum ferritin, transfusion burden, enhanced LPI (defined as biologically active iron) and LIC (determined by MRI) in a cohort of hematopoietic malignancies (92 AML and 20 MDS patients) undergoing HSCT. The results indicated that LIC values more than three times the upper limit of normal were significantly associated with increased non-relapse mortality. It is still a possibility that this impact could be restricted to patients having undergone a reduced-intensity conditioning regimen (this case concerns 83% of the studied patients). Moreover, the ALLIVE study showed a significantly increased incidence of non-relapse mortality in patients exhibiting a raised baseline enhanced-NTBI concentration. Moafi *et al.* (77) concluded that high levels of bone marrow iron were associated with a poor response to treatment and to the risk of relapse.

To summarize, body iron overload assessed by direct methods has a predictive value for poor survival after HSCT. However, the predictive value of increased serum ferritin levels, which reflect not only body iron excess but also other mechanisms, of which the foremost is inflammation, may be stronger.

577

578 4.2. The impact of iron removal during leukemia treatment

579 4.2.1. Iron chelation

580 We will focus on the results obtained using oral iron chelation, especially deferasirox (DFX). Sivgin
581 *et al.* (148) retrospectively investigated 80 patients including 45 AML and 18 ALL patients for whom
582 the pretransplant serum ferritin levels were ≥ 1000 ng/mL. Thirty-seven patients were given DFX
583 and compared to 26 patients who were phlebotomized due to DFX side effects or compliance
584 problems. Overall survival and disease-free survival were significantly better in the DFX group. The
585 first prospective multicenter clinical trial of DFX in adult allogeneic HSCT was carried out in Spain
586 (149). Thirty patients (including 17 AML patients and one ALL patient), with transfusional iron
587 overload (serum ferritin ≥ 1000 ng/mL or ≥ 20 units of packed red blood cells; the LIC assessment
588 was assessed by MRI, depending on equipment availability) received DFX at a starting dose of 10
589 mg/kg for 52 weeks or until the serum ferritin level was less than 400 ng/mL on two consecutive
590 occasions. There were no severe drug-related adverse events. A significant reduction in ferritinemia
591 was observed from baseline to 52 weeks (1444 to 755 ng/mL) in the intent-to-treat population. LIC
592 was also significantly reduced for the seven patients for whom basal and final LIC (at 52 weeks)
593 could be obtained. The multicenter German study (DE02) (150) assessed the safety and efficacy of
594 DFX in 76 recipients of allogeneic HSCT (comprising 52 AML) who started at a dose of 10 mg/kg with
595 an escalating design up to 20 mg/kg. The median exposure was 330 days. Seventy-one percent of
596 the patients experienced drug-related adverse events (increased blood creatinine, nausea and
597 abdominal discomfort) leading to occasional discontinuation. The median compliance rate was >
598 80%. A significant decline in serum ferritin was observed (from 2045 to 957 ng/mL) and a negative
599 iron balance was obtained in 84% of patients.

In summary, these studies indicate that DFX is effective for iron chelation therapy after HSCT, with a manageable safety profile. Gastrointestinal side effects may be reduced with the new DFX film-coated tablet formulation (151, 152).

4.2.2. Phlebotomy

Iron removal by phlebotomy is the alternative to iron chelation for therapeutic iron removal. As to the impact of phlebotomies in leukemia patients, most studies have involved patients after allogeneic HSCT. We will focus here on those studies that used a direct evaluation of tissue iron load by liver biopsy (153, 154) or imaging techniques (MRI (155) and, in one study, SQUID (superconducting quantum interference device) (156)). All of the studies converged to conclude that phlebotomy was a safe and well-tolerated procedure. A significant reduction in the liver iron concentration (and serum ferritin) was obtained. A single retrospective study reported that DFX was more effective on iron excess than phlebotomy. However, only serum ferritin levels were evaluated (148).

On the whole, further clinical studies should be carried out to ascertain if the long-term prognosis after HSCT is favorably influenced by decreasing iron excess, through chelation or phlebotomy. Irrespective of the therapeutic method, it should be emphasized that it is highly recommended to avoid foods containing high amounts of iron since this is a natural and cheap way to counteract intestinal iron absorption.

CONCLUSION

Close functional interactions exist physiologically between iron and bone marrow that concern the leukocyte lineage as well as the erythroid cells. From a pathological point of view, iron acts as a co-carcinogenic factor that experimentally promotes leukemia cell growth, whereas clinical data promoting the role of iron in MDS transformation is still under debate. Body iron overload, best

assessed by direct MRI quantification, is usually not increased at the time of leukemia diagnosis; it increases significantly during medical care, especially due to multiple transfusions. Iron overload, notably through the damaging effects of NTBI, which is more likely to appear in the plasma with chemotherapy, significantly contributes to a poor prognosis after HSCT. Hyperferritinemia only partially reflects excess iron in the body but seems to be an interesting prognostic factor since it also occurs in inflammation. Iron removal, mostly by oral chelation, must become the standard of care whenever significant body iron overload has been firmly established.

FUTURE CONSIDERATIONS

In our opinion, increased awareness of the importance of diagnosing body iron excess in leukemia patients remains a key objective. Further clinical trials evaluating the long-term prognostic effect of iron removal by oral chelation are warranted, in principle, but may raise practical difficulties for recruiting patients. Strategies to target iron removal from specific tissues should be considered.

PRACTICE POINTS

- Iron experimentally promotes leukemia cell growth; however, clinically, the promoting role of iron overload in the transformation of MDS into leukemia is still being debated.
- The assessment of body iron status in leukemia patients requires the direct visualization and quantification of iron excess, especially by magnetic resonance imaging (MRI).
- Iron overload, mainly due to transfusions, is likely to contribute to a poor prognosis after hematopoietic stem cell transplantation.
- Hyperferritinemia is an interesting overall indicator of a poor prognosis; this parameter reflects the inflammatory status and, albeit partially, the body iron burden.
- Iron removal by oral chelation or phlebotomies is well tolerated and acts efficient on the body iron burden; its favorable effect on the long-term prognosis is uncertain.

RESEARCH AGENDA

- Further clinical trials investigating the impact of iron on MDS transformation, with appropriate measurement and follow-up of iron burden.

- Exploration of the promoting role of iron in the development of myelodysplastic/myeloproliferative syndromes from hematopoietic stem cells especially by using appropriate transgenic mouse models.

CONFLICT OF INTEREST

PB has been an occasional consultant and member of advisory board for Novartis. No links of interest for the other authors in the frame of the present article.

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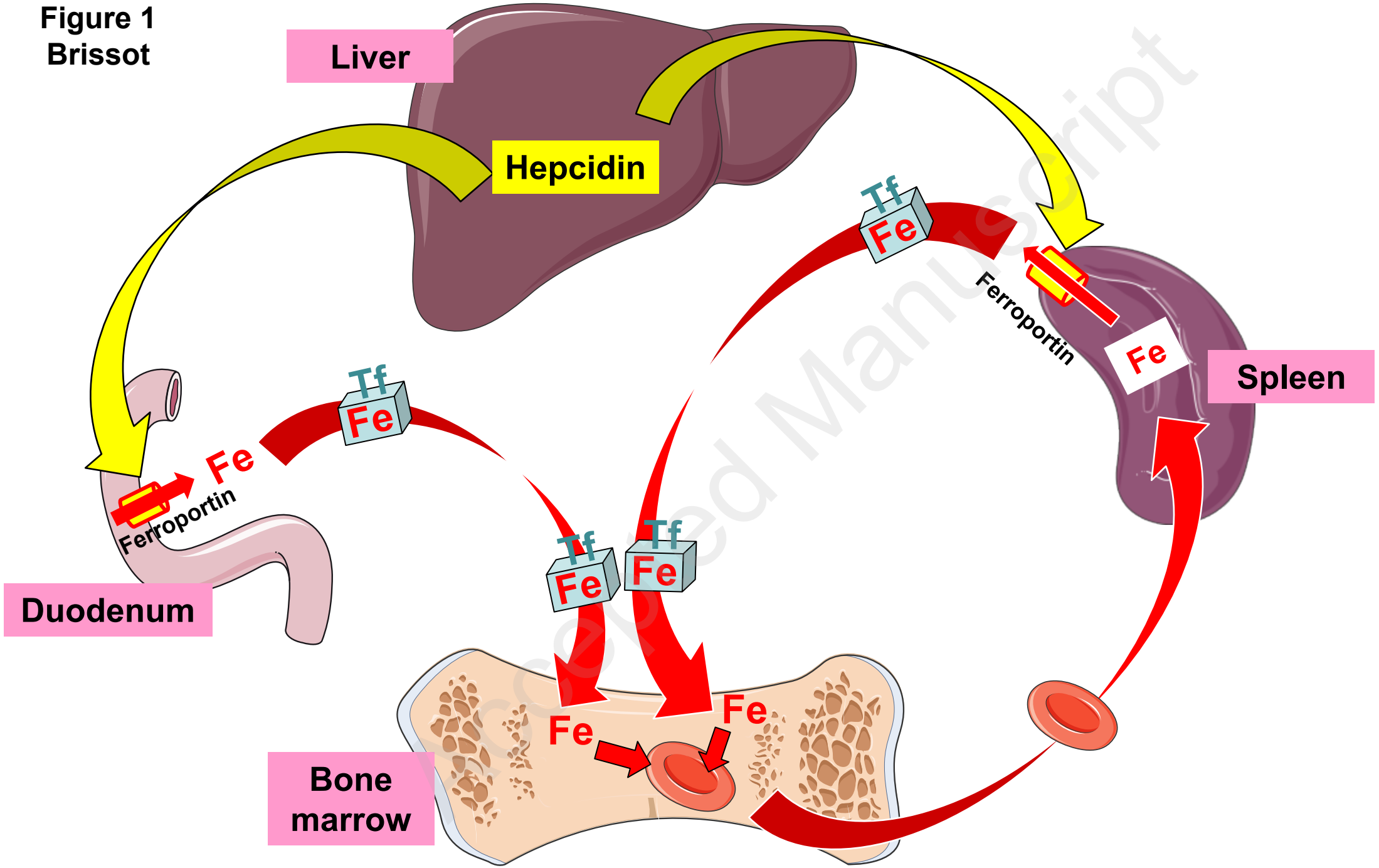
FIGURE LEGENDS

FIGURE 1. Systemic iron homeostasis. Plasma iron comes from the duodenum (digestive iron absorption) and the spleen (iron originating from erythrophacocytosis). Once in the plasma, iron is bound to transferrin (TF) and mainly sent to the bone marrow for the production of new red blood cells. The "bone marrow-spleen-bone marrow" cycling process represents, quantitatively, every day, about 20 times the amount of iron coming from digestive absorption. If plasma iron increases, hepcidin production by the liver is increased, leading to decreased activity of ferroportin both at the duodenum and spleen levels, which in turn decreases plasma iron (the reverse regulation occurs in case of decreased plasma iron).

FIGURE 2. Bone marrow and iron metabolism. Due to iron toxicity, malignancy or chemotherapy, the functionality of bone marrow stem cells (BMSC) can be altered, leading, for the erythroid cell lineage, to dyserythropoiesis, which in turn causes an increased production of various factors including GDF15 and the hormone erythroferrone. These factors lead to a decrease in hepcidin production, then to an increase of plasma iron and, through non-transferrin bound iron, to iron deposition into various parenchymal cells (in first line the hepatocytes): it may lead to body iron overload (this iron excess can increase hepcidin but the increasing signal remains dominated by the reverse impact of dyserythropoiesis (and anemia itself)).

Figure 1
Brissot



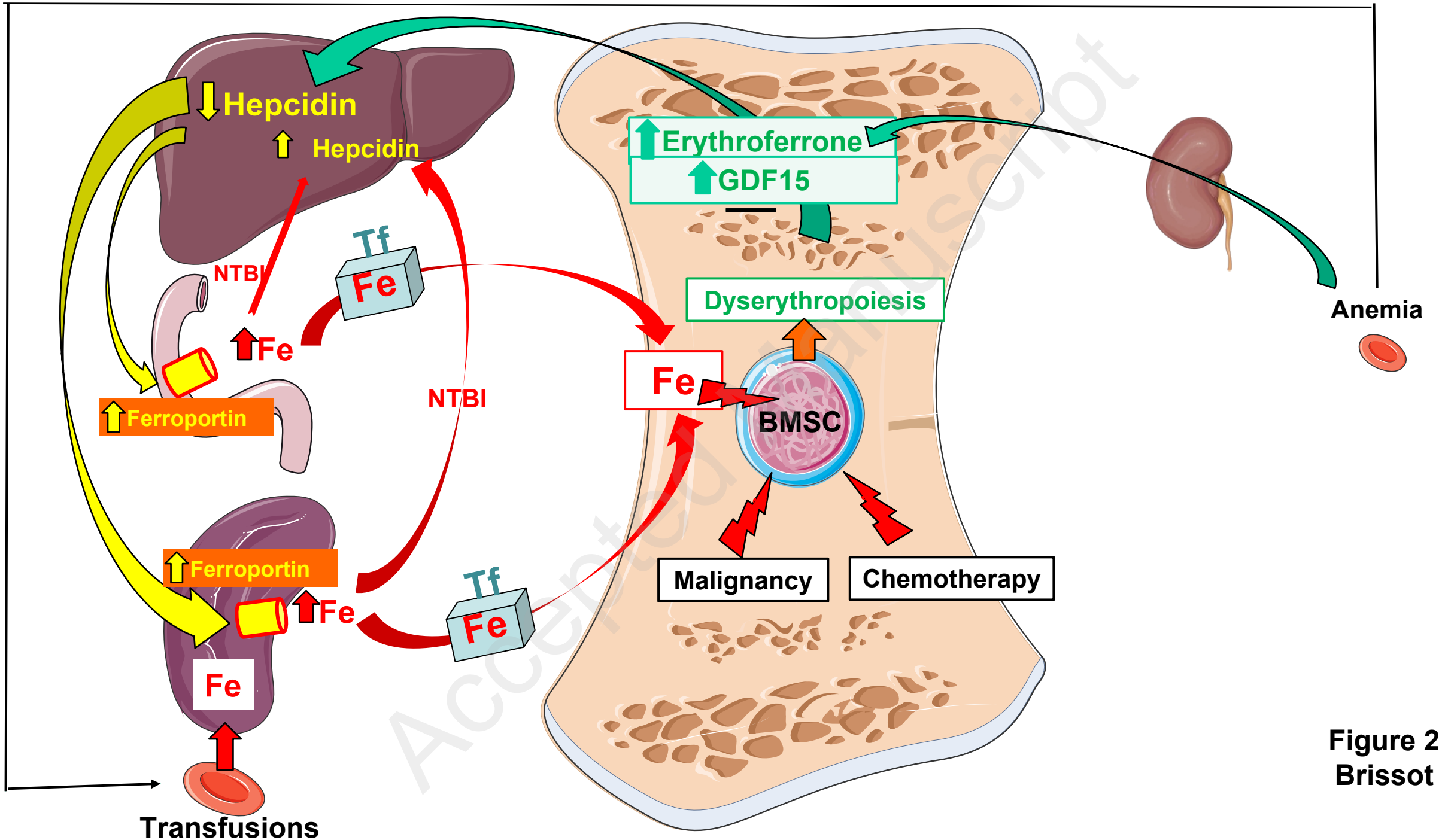


Figure 2
Brissot

Gene name and aliases	Gene symbol (Official, others)
aconitase 1, soluble	*ACO1 ; IRP1 ; IREB1; IREBP*
ceruloplasmin	*CP*
erythroferrone	*ERFE, FAM132B*
ferritin, heavy polypeptide 1	*FTH1 ; FTH*
ferritin, light polypeptide	*FTL ;* L-ferritin
ferroportin ; solute carrier family 40 (iron-regulated transporter), member 1	*SLC40A1 ; IREG1 ; MTP1; SLC11A3; FPN*
hemochromatosis	*HFE ; HH; HFE1; HLA-H*
hepcidin ; human anti-microbial peptide	*HAMP ; HEPC ; LEAP1*
hephaestin	*HEPH ; CPL*
iron-responsive element binding protein 2	*IREB2 ; IRP2 ; ACO3; IRP2AD*
thrombopoietin Receptor	MPL, TPO-R
transferrin	*TF*
transferrin receptor	*TFRC ; TFR1 ; TFR; CD71*
transferrin receptor 2	*TFR2*

Table 1 : Principal iron regulatory genes presented in the review, with their multiple gene symbols. We have underlined the names and/or symbols used in the review.