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# Detailed investigation of erythropoiesis and iron metabolism in the context of allogeneic hematopoietic stem cell transplantation

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# Table of contents

# Abbreviations and acronyms

ACD : anemia of chronic disease

ActRIIA : activin type IIA receptor

AKT : protein kinase B

AL : acute leukemia

ALK : Activin-Like Receptor

Allo-HSCT : allogeneic hematopoietic stem cell transplantation

AML : acute myeloid leukemia

ASAT : aspartate amino transferase

ATG : Anti-thymocyte globulin

Auto-HSCT : autologous hematopoietic cell transplantation

Bcl-xL : B-cell lymphoma-extra-large

BFU-E : burst-forming unit-erythroid

BMP : bone morphogenetic protein

BMPR : bone morphogenetic protein receptor

BM(T) : bone marrow (transplantation)

BRE : bone morphogenetic protein responsive element

CBP : CREB-binding protein

CFU-E : colony-forming unit-erythroid

CMV : cytomegalovirus

CREBH : cyclic AMP response element-binding protein H

CRP : C-reactive protein

DA : darbepoetin alpha

DLI : donor lymphocyte infusion

DMT1 : divalent metal transporter-1

EB : erythroblasts

EBMT : European Group for Blood and Marrow Transplantation

EGF : Epidermal Growth Factor

EMP : erythroblast macrophage protein

EPO : erythropoietin

EPO-R : EPO receptor

ER : endoplasmic reticulum

ERK : extracellular signal-regulated kinases

ERFE : erythroferrone

ESA : erythropoiesis-stimulating agents

Fas-L : Fas ligand

FLVCR1 : Feline Leukemia Virus Subgroup C Receptor 1

FID : functional iron deficiency

FIH-1 : hypoxia-inducible factor 1-alpha inhibitor

Flt-3L : Fms-related tyrosine kinase 3 ligand

GATA : zinc finger transcription factor

GM-CSF : granulocyte-monocyte colony-stimulating factor

GDF-15 : growth differentiation factor 15

GLMM : generalized linear mixed model

GVHD : graft-versus-host disease (acute GVHD : aGVHD; chronic GVHD : cGVHD)

H : heavy

HAMP : Hepcidin anti-microbial peptide gene

Hb : hemoglobin

HCP1 : heme cell transport protein

Hct : hematocrit

HCT : hematopoietic cell transplantation

HCT-CI : hematopoietic cell transplantation – comorbidity index

HIF : hypoxia-inducible factor

HJV : hemojuvelin

HLA : Human Leukocyte Antigen

HMOX1 : heme oxygenase 1

HMOX2 : heme oxygenase 2

HR : hazard ratio

HRE : hypoxia-responsive element

HRG1: Heme Responsive Gene

HSC : hematopoietic stem cell

HSCT : hematopoietic stem cell transplantation

ICAM-4 : InterCellular Adhesion Molecule 4

IDA : iron deficiency anemia

IFN-γ : interferon-gamma

IL-6 : interleukin-6

IL-3 : interleukin-3

IL-3R : IL-3 receptor

IL-11 : interleukin-11

IO : iron overload

IP : interstitial pneumonia

IPPS : International Prognostic Scoring System

IRE : iron regulatory element

IRP : iron regulatory protein

IV : intravenous

JAK2 : Janus kinase 2

L : light

LDH : lactate dehydrogenase

LEAP-1 : liver-expressed antimicrobial peptide 1

LIC : liver iron content

LIP : labile iron pool

LPS : lipopolysaccharide

LSEC : liver sinusoidal endothelial cells

MA : myeloablative

MAPK : mitogen-activated protein kinase p38

MC : macrophage

MCV : mean corpuscular volume

MDS : myelodysplastic syndrome

MHC : major histocompatibility complex

MMF: mycophenolate mofetil

MPN : myeloproliferative neoplasm

MRD : minimal residual disease

MRI : magnetic resonance imaging

mRNA : messenger ribonucleic acid

MTX : methotrexate

NCOA4 : nuclear receptor coactivator 4

NF-κB : nuclear factor κB

NMA : non-myeloablative

NRM : non-relapse mortality

NTBI : non-transferrin bound iron

OR : Odds ratio

OS : overall survival

P300 : histone acetyltransferase p300

PAM : pretransplant assessment of mortality

PAMP : pathogen-associated molecular pattern

PCBP : poly(rC)-binding proteins

PBSC : peripheral blood stem cell

PS : performance status

PFS : progression-free survival

PHD : prolyl hydroxylase

PI3K : phosphatidylinositol 3-kinase

p-JAK2 : phosphorylated Janus kinase 2

PRCA : pure red cell aplasia

$R^2$ : r-square value

RBC : red blood cell

RIC : reduced-intensity conditioning

RNA : ribonucleic acid

RME : receptor -mediated endocytosis

rHuEPO : recombinant human erythropoietin

SCD : sickle cell disease

SCF : stem cell factor

Slc11a2 : solute carrier family 11 member 2

SD : standard deviation

SE : standard error

SeFe : serum iron

SF : serum ferritin

SMAD : son of mothers against decapentaplegic

SSRIs : selective serotonin reuptake inhibitors

STAT : signal transducer and activator of transcription

STAT-RE : STAT Responsive Element

STAT3-RE : STAT3-Responsive Element

sTfR : soluble transferrin receptor

TAD : transactivation domain

TBI : total body irradiation

Tf : transferrin

TfR : transferrin receptor

TGF : transforming growth factor

TLR : toll-like receptor

TNF : tumor necrosis factor

Tpo : thrombopoietin

TRAIL : TNF-related apoptosis-inducing ligand

TTR : transthyretin

Tsat : transferrin saturation

TWGS1 : twisted gastrulation BMP signaling modulator 1

UCB : umbilical cord blood

VCAM1: Vascular Cell Adhesion Molecule 1

VHL : von Hippel Lindau

VOD : veno-occlusive diseas

WBC : white blood cells

# I INTRODUCTION

# 1. Erythropoiesis and iron

## 1.1 Erythropoiesis

### 1.1.1 Erythropoietic progenitors and precursors

Two million mature red blood cells (RBC) or erythrocytes are produced every day by a continuous process called erythropoiesis. The cells involved in erythropoiesis are the erythropoietic progenitors and precursors that derive from hematopoietic stem cells (HSCs), located in the bone marrow (BM) (1, 2) . These cells go through a series of developmental stages before becoming RBC that have an average lifespan of 120 days and the ability to transport oxygen throughout the body.

HSCs are pluripotent cells capable of self-renewal, but also of differentiating into all blood cell types by evolving into the myeloid or lymphoid lineage. The choice of the lineage to which HSCs commit depends on signals and growth factors received by the BM. Erythropoietin (EPO), among other factors, is key for the development of RBC from HSCs (3, 4).

Erythroid differentiation begins with the engagement of a HSC into the myeloid lineage, starting with a common progenitor, capable of giving rise to both RBC and platelets (5). This progenitor, at the next step, becomes an early erythroid progenitor called “burst-forming unit-erythroid” (BFU-E). It is only at the BFU-E stage that the cells are irreversibly committed to the pathway of RBC differentiation. Then, the BFU-E gives rise to a colony of cells called “colony-forming unit-erythroid” (CFU-E) (1).

CFU-E are the first recognizable erythroid precursors. While BFU-E cells have a slow proliferation rate, CFU-E progenitors divide rapidly, forming small colonies (up to 64 erythroblasts) within a few days in methylcellulose cultures. Five to eight times more CFU-E than BFU-E cells can be observed in the BM (6).

Erythroid precursors are the intermediate cells between erythropoietic progenitors and mature RBC. They undergo several cycles of division and differentiation, each characterized by changes in cell size, shape, and staining properties. The main stages of erythroid precursors are proerythroblasts, basophilic erythroblasts, polychromatic erythroblasts and orthochromatic erythroblasts evolving into reticulocytes. From the basophilic erythroblast stage, hemoglobin (Hb) is produced thanks to the strong expression of transferrin receptors (TfR) allowing the acquisition of large amounts of iron. The progressive accumulation of Hb in the cytoplasm leads to changes in cytoplasmic appearance. The last cell division takes place at the polychromatophilic stage.

Finally, the nucleus is expelled when erythroblasts are sufficiently mature (orthochromatic erythroblast stage), and reticulocytes appear in the circulation while continuing active Hb production. At this stage, the cell is filled with Hb, which gives it its characteristic red color. It takes

about a week for reticulocytes to develop into erythrocytes in healthy individuals (1). As reticulocytes mature, ribonucleic acid (RNA), mitochondria and ribosomes are degraded and protein synthesis ceases (7). Mature erythrocytes are shaped like a biconcave disc and contain high concentrations of Hb, which binds and transports oxygen **(Figure 1).**

Erythropoiesis is thus a continuous process (including cell proliferation and differentiation). Furthermore, this production can be increased up to 20-fold in anemia, including through a decrease in apoptosis of erythroid progenitors (2, 3, 8).

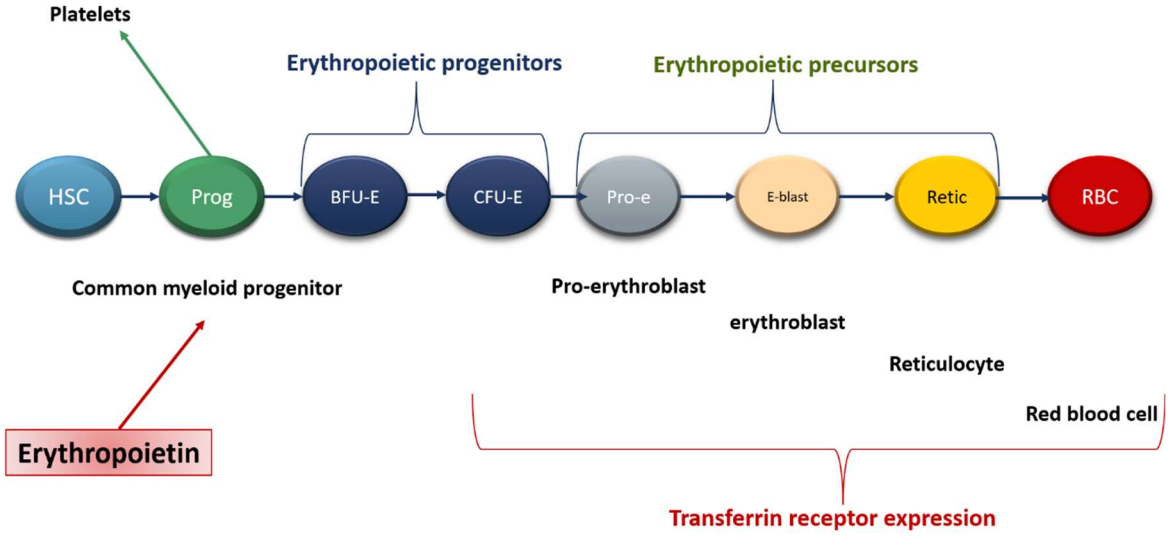


***Figure 1:* Erythropoietic progenitors and precursors**. *HSC: hematopoietic stem cell; Prog: progenitor cell; BFU-E: burst-forming unit-erythroid; CFU-E: colony-forming unit-erythroid; Pro-e: pro-erythroblast; E-bast: erythroblast; Retic: reticulocyte; RBC: red blood cell.*

### 1.1.2 Regulation of erythropoiesis

Erythropoiesis is influenced by the environment. The organism adapts the production of RBC according to environmental factors (high altitude) and mechanical factors (sports).

At the cellular level, erythropoiesis is a dynamic process regulated by various cytokines and growth factors that influence cell survival, proliferation, and differentiation. During erythropoiesis, HSCs proliferate and differentiate into mature erythrocytes.

Several parameters influence erythroid differentiation positively or negatively. These parameters are physical (involving cell-cell or cell-extracellular matrix interactions), chemical (cytokines, growth factors) or genetic. Most growth factors are produced within the marrow environment consisting of stromal cells, endothelial cells, hematopoietic cells, osteoblasts, and the extracellular matrix (1, 2).

EPO is the main growth factor that stimulates erythropoiesis and is therefore described in more detail in the following chapter. Other growth factors involved in the regulation of erythropoiesis have also been identified: hypoxia-inducible factors (HIF), stem cell factor (SCF), Fms-related

tyrosine kinase 3 ligand (Flt-3L), thrombopoietin (Tpo), interleukin-11 (IL-11), interleukin-3 (IL-3), and granulocyte-monocyte colony stimulating factor (GM-CSF) (1, 2, 7).

***Erythroblast islands*** that regulate erythroid differentiation in the BM were described in 1958 by Marcel Bessis (9) **(Figure 2)**. They consist of a central macrophage (MC) surrounded by five to thirty erythroblasts at different stages of maturation. Although erythroid differentiation is possible in vitro without the need for co-culture with these macrophages, only interaction with macrophages can achieve proliferation and differentiation rates that allow the generation of approximately two million reticulocytes per second under normal erythropoietic conditions (10).

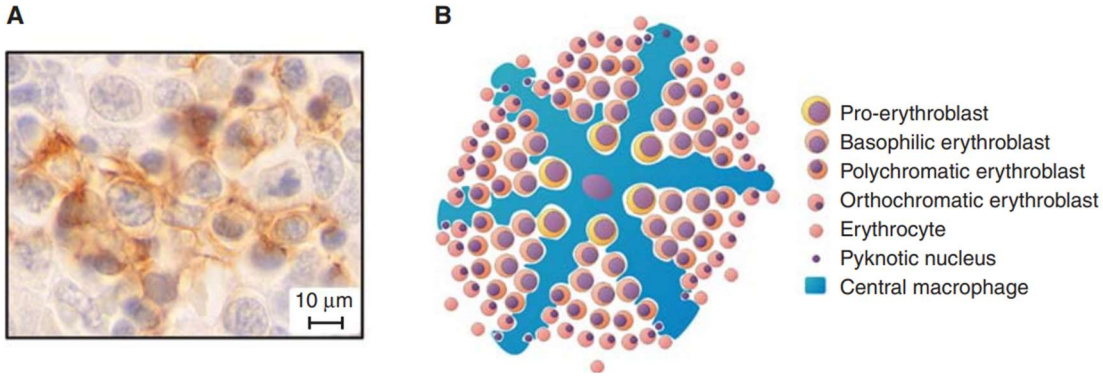


*Figure 2:* ***The erythroblastic island.*** *(A) Erythroblastic island in fetal liver. The cytoplasmic extensions of the central macrophage (stained with the F4/80 antibody) (brown) are surrounding erythroid cells at various stages of differentiation. (B) Schematic drawing of an erythroblastic island. Figure from Cold Spring Harb Perspect Med 2013 (1)*

***The glycoprotein erythroblast macrophage protein (EMP)*** is expressed by erythroblasts (EB) and MCs, which can interact through homophilic binding. EMP therefore mediates erythroblast-macrophage interaction during erythroid differentiation but is also essential for erythroid proliferation and terminal differentiation. Indeed, blocking EMP with an anti-EMP antibody induces a decrease in EB proliferation, maturation and enucleation, associated with an increase in apoptosis (11). Mice invalidated for this protein die soon after birth and suffer from severe anemia (12). Although EMP was the first protein identified in these EB/MC interactions, other protein pairs have since been found; Integrin α4β1 expressed on the surface of EB interacts with the VCAM1 (Vascular Cell Adhesion Molecule 1) protein expressed by MCs. Inhibition of this interaction by antibodies disrupts islet formation and reduces the number of islets (13). Another pair of proteins has been identified, i.e. the ICAM-4 (InterCellular Adhesion Molecule 4) (EB) / αv (MCs) pair. Blocking the interaction between these two proteins also reduces the number of islets by 70% (14). A recent study showed, in a mouse model invalidated for agrin α5β1 expressed by Ebs and MCs, a decrease in the number of Ebs by 30-60% as well as anemia and disorganization of islets visible by confocal microscopy (15).

Different cytokines are involved in the initial phase of HSC differentiation into erythroid progenitor cells and in the late phase of cell division towards mature erythroid cells (16) (**Figure 3).** These cytokines are pivotal in governing normal hematopoiesis, necessitating an equilibrium between hematopoietic growth factors and myelosuppressive factors to ensure optimal generation of diverse hematopoietic cell lineages. ***Negative regulation of erythropoiesis,*** under the control of a complex network of transcriptional factors such as transforming growth factor (TGF)-β1, tumor necrosis factor (TNF)-α, interferon-gamma (IFN-γ), and Fas/Fas-ligand (FasL) and TNF-related apoptosis-inducing ligand (TRAIL), also occurs within the EB islet (17). Consequently, the low rate of inefficient erythropoiesis under basal conditions is partly explained by apoptotic mechanisms.

The primary phases of erythropoiesis are regulated by ***SCF/stem cell growth factor receptor (c-Kit)***, ***IL-3/IL-3 receptor*** (IL-3R), and ***GM-CSF*** (1, 18). Indeed, SCF is a cytokine that binds to its receptor KIT, a tyrosine kinase receptor, and triggers a signaling cascade that regulates the differentiation of erythroid progenitor cells by activating multiple signaling pathways, including PI-3 kinase, Src kinases, and PLC-g (19).

Zinc finger transcription factors (GATA), ***GATA-1 and GATA-2***, are the major transcriptional regulatory factor of erythropoiesis (20, 21). GATA-2 is expressed in early while GATA-1 is more present in late stages of erythropoiesis (22). GATA-2 supports erythroid proliferation and reduces erythroid differentiation (23). Inversely, GATA-1 promotes erythroid differentiation at the end of erythropoiesis by up-regulating specific red lineage genes. GATA-1 also induces the expression of the anti-apoptotic protein B-cell lymphoma-extra-large (Bcl-xL) and the erythropoietin receptor (EPO-R) with the transcription factor signaling and activator of transcription 5 (STAT5) (23, 24). Caspases, which are a family of cysteine proteases that play key roles in apoptosis, can cleave GATA-1 during erythropoiesis. This cleavage of GATA-1 results in the release of a truncated GATA-1 protein, thereby stopping erythroid differentiation and generating cell death (22). Heat shock protein 70 (Hsp70) is a chaperone protein that helps to protect cells from stress in response to various cellular signals. Hsp70 is upregulated during erythropoiesis, and that it plays a role in protecting erythroid cells from oxidative stress. Additionally, Hsp70 can protect cells from apoptosis by preventing the cleavage of GATA-1 by caspase-3 (25).

Interferons (IFN-α, IFN-β, and IFN-γ), TGF-β, and TNF-α can all activate the mitogen-activated protein kinase p38 (MAPK) in primary human hematopoietic progenitors. The activation of p38 is necessary for the myelosuppressive action of these cytokines on hematopoiesis (26). Moreover, mature Ebs can inhibit the survival and differentiation of immature Ebs through ***Fas/Fas-L interactions (27).*** Fas and its ligand (Fas-L) belong to the TNF and TNF receptor families, respectively. In addition to its well-known immune functions, the Fas/FasL system may be involved in EB apoptosis (28). Fas is expressed by all Ebs and especially the most immature cells

(proerythroblasts and basophilic erythroblasts) and can be stimulated by IFN-γ (16, 17, 27, 29). It has been proposed that mature Fas-L+ erythroblasts, through a negative feedback regulatory mechanism, kill immature Fas+ erythroblasts, and that macrophages are responsible for the clearance of dead erythrocytes (30). Therefore, Fas-L expressed by mature erythroblasts will bind to Fas expressed by immature erythroblasts, triggering apoptosis pathways, thus preventing overproduction of RBC (28, 30). This implies the activation of downstream caspases, the cleavage of GATA-1, a key factor in erythroid differentiation, inducing apoptosis of excess erythroblasts (30). This Fas/Fas-L pathway also cleaves ***TAL-1 (T-cell Acute lymphocytic Leukemia protein 1,*** also known as the stem cell leukemia (SCL) gene). TAL-1 encodes a basic helix-loop-helix protein that is essential for the formation of all hematopoietic lineages, including primitive erythropoiesis (31). In particular, the activation cascade induced by the Fas/Fas-L interaction leads to an arrest of erythroid maturation by activating the cleavage of GATA-1 and TAL-1 (27, 32).

More immature progenitors are protected from the FAS pathway because they express c-FLIP, an inhibitor of Fas (33). Fas-L is expressed by mature orthochromatic erythroblasts that are insensitive to Fas signaling, as if Fas was not related to apoptotic mechanisms in mature Ebs. The action of Fas-L on immature Ebs is greatly diminished at high concentrations of EPO, allowing activation of erythropoiesis (17, 27). Indeed, the expansion of Ebs in response to stress is proportionally mediated by EPO through suppression or downregulation of Fas/Fas-L expression in Ebs (28, 34). However, Fas receptors can bind to Fas-L overexpressed by certain tumor cells, such as malignant plasma cells in multiple myeloma, leading to paraneoplastic anemia (35).

Nevertheless, lpr mutant mice carrying a defective Fas gene do not exhibit major abnormalities in erythroid development (36). Moreover, inhibition of IFN-γ-induced erythroid apoptosis was only partially restored after blocking the Fas pathway (37). These findings suggest that apoptotic pathways, other than Fas/Fas-L, are involved in the control of erythropoiesis. Indeed, another regulator demonstrated in erythropoiesis is *TRAIL*. TRAIL, also known as Apo-2L, is a proapoptotic member of the TNF family first identified by Wiley in 1995 (38) and described to induce apoptotic cell death in various cancers and infected cells expressing death receptor-4 or -5 (39, 40). Unlike Fas, TRAIL is toxic to many cancer cells but spares most normal cells, and some Fas-resistant cell lines remain sensitive to TRAIL-induced apoptosis (41). Primarily secreted by cells of innate immunity or adaptive immunity such as NK cells, T lymphocytes, or macrophages, TRAIL is crucial in innate and adaptive immune responses (42). Its production is triggered by pro-inflammatory cytokines such as interferon, notably IFN-γ (43).

TRAIL shares similarities with Fas, such as the negative effect on erythropoiesis by blocking differentiation and promoting apoptosis (44-46). The signaling pathway between TRAIL and its receptor would notably pass through the activation of caspases and the induction of ERK1/ERK2 signaling (47). Furthermore, TRAIL also regulates the maturation of myeloid cells (48) and

eliminates senescent neutrophils (49). TRAIL receptors are detectable in the early phases of erythroid development, and are less expressed on mature Ebs, which display more TRAIL than immature Ebs. Peripheral blood monocytes/macrophages have been identified as a potential source of TRAIL in the bone marrow, producing this protein in response to certain signals such as IFN-γ or IFN-α (50). Furthermore, Fas/FasL and TRAIL could be effectors of IFN-γ to inhibit erythroid precursor growth and differentiation (29). On the other hand, the addition of EPO has been found effective in counteracting erythroid cell apoptosis induced by both TRAIL and Fas-L (45). Thus, macrophages may play a central role in the regulation of erythropoiesis by influencing the balance between survival factors like EPO and apoptosis inducers such as TRAIL and Fas-L.

The terminal phase of erythropoiesis is dependent on iron metabolism and is regulated primarily by EPO and SCF (51, 52). Actually, SCF synergizes with EPO in stimulating the proliferation and expansion of developing erythroid precursors and may play a role in the phosphorylation of the EPO receptor itself (19). In addition, vitamin B12 and folic acid, copper, zinc and iron are essential for erythroid maturation (53). Erythroid differentiation and maturation are also negatively regulated by members of the transferrin (Tf) group and its cellular receptor and by TGF and the TGF-β superfamily (54, 55).

Another stimulator of erythropoiesis is serotonin. In addition to its role as a neurotransmitter, serotonin is also produced and utilized in peripheral organs (56). Fouquet *et al.* demonstrated that serotonin synthesized in the bone marrow is crucial for the survival and proliferation of erythroid progenitors (57). Their research unveils a novel role of selective serotonin reuptake inhibitors (SSRIs) collaborating with G-CSF in enhancing the recovery of all three hematopoietic lineages after autologous hematopoietic stem cell transplantation (HSCT) in patients and after sublethal irradiation in mice. However, in the absence of stress-induced hematopoiesis, SSRIs do not appear to affect blood cell production.

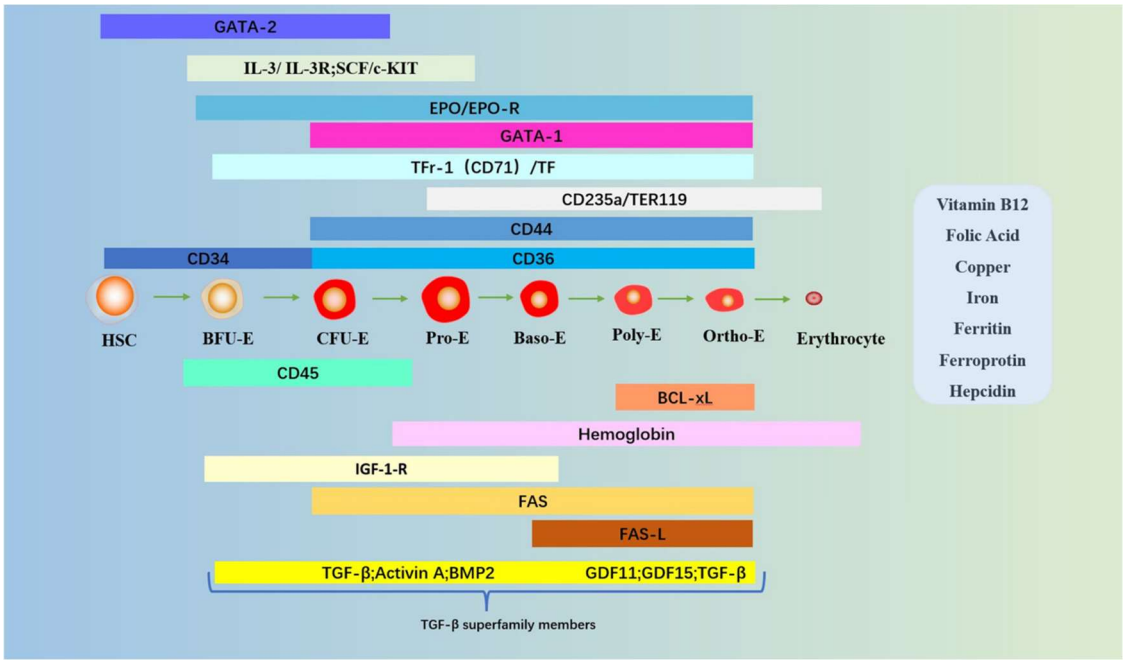


***Figure 3:*** ***The main pathways and molecules involved in regulating erythropoiesis****. The different stages are shown: HSC, BFU-E, CFU-E, Pro-E, Baso-E, Poly-E, Ortho-E, and erythrocytes. Molecules involved: zinc finger factors that bind GATA sequences (GATA-1, GATA-2); IL-3; IL-3-R; SCF; c-Kit; EPO; EPO-R; Ter-119, glycophorin A-associated protein; CD235a, glycophorin A; CD44, cell surface adhesion molecule; CD34, transmembrane phosphoglycoprotein; CD36, platelet glycoprotein protein 4; CD45, common marker of leukocytes; BCL-xL, anti-apoptotic protein; hemoglobin; FAS; FAS-L; Tf; TfR-1 (or CD71), transferrin receptor 1; TGF-β; activin A; BMP-2, bone morphogenetic protein 2; GDF, growth differentiation factor. Vitamins, trace elements, and iron metabolism proteins necessary for erythropoiesis: vitamin B12, folic acid, copper, iron, ferritin, ferroportin, hepcidin). Figure and legend from Hao Zhang et al (16).*

## 1.1.3 Erythropoietin and erythropoiesis-stimulating agents

After Deflandre and Carnot in 1906 identified a serum “substance” capable of stimulating red cell production, more than 50 years of effort led to the identification of EPO in 1977 when Goldwasser and Kung purified the protein (58), and that of its receptor (EPO-R) in 1989.

### *1.1.3.1 Structure and production of EPO*

EPO is a 30,400 dalton glycoprotein composed of 165 amino acids. It originates from a polypeptide encoded by the EPO gene itself discovered in 1985, and then cleaved and glycosylated (19, 59). Throughout embryonic life, EPO is produced by neural crest- and neuroepithelium-derived cells to promote erythrocyte differentiation in the yolk sac (60, 61). During fetal life, hepatocytes and interstitial cells around their central vein in the liver become the primary sites of EPO production. This hepatic production continues minimally and provides 10-20% of EPO during adult life while the main production then takes place in the peritubular cells of the kidney, characterized by FOXD1 expression (62-65). EPO can be translated in small amounts in the spleen, BM, lung, and brain, but it does not replace the necessary erythropoietic regulation by the kidney (66-69). Studies have also suggested EPO production by osteoblasts via HIF activation (70, 71).

### *1.1.3.2 Regulation of EPO production*

Oxygen-sensing mechanisms are based on tissue oxygen concentration (3). Both high oxygen consumption and limited oxygen supply result in oxygen gradients in the renal cortex because oxygen can diffuse directly from arteries to veins (due to their proximity in the architecture of the kidney) prior to the arrival of blood in tubular capillaries (3).

EPO expression is primarily regulated by HIF, a heterodimer between an $O_2$-dependent α subunit (HIF-α : HIF-1α, HIF-2α, or HIF-3α), and a constitutively expressed β subunit (HIF-1β). HIF-2α is particularly associated with EPO regulation (72, 73).

Under normal oxygenation conditions, prolyl hydroxylase enzymes (PHD1, PHD2, and PHD3 proteins) hydroxylate specific proline residues on the HIFα protein, which enables its recognition by the von Hippel-Lindau (VHL) protein, a component of an E3 ubiquitin ligase complex that targets HIFα for degradation via the ubiquitin-proteasome system (74, 75) **(Figure 4)**. This degradation pathway is dependent on the availability of oxygen and iron, as PHD enzymes require both oxygen and iron as cofactors to catalyze hydroxylation of HIF-α (64, 76, 77). Mutations in VHL, PHD2 and HIF-2α have been identified in patients with familial EPO-dependent erythrocytosis (78-80).

Reduced blood oxygen levels, or hypoxia, is the primary stimulus for increased EPO expression (63, 81). Under hypoxic conditions, the availability of oxygen for hydroxylation of HIF-2α by PHD enzymes is limited. As a result, the HIF-2α protein becomes stabilized and translocated to the nucleus, where it forms a heterodimer with HIF-1β. The HIF-2α-HIF-1β complex then binds to hypoxia-responsive elements (HREs) in the promoter/enhancer region of the EPO gene, recruiting coactivators such as the histone acetyltransferase p300 (p300) and CREB-binding protein (CBP) to enhance transcriptional activation of the HIF complex (82) **(Figure 4).** The HIFα transactivation domain (TAD) is a critical component for the recruitment of coactivators such as p300 and CBP (83). This domain interacts with p300/CBP through protein-protein interactions, which leads to the acetylation of histone proteins and chromatin remodeling in the vicinity of the EPO gene promoter (84). This process ultimately enhances the accessibility of the EPO promoter to RNA polymerase II and the general transcriptional machinery, resulting in increased EPO messenger ribonucleic acid (mRNA) synthesis and subsequent erythropoiesis.

The transcriptional activity of HIFα subunits can also be negatively regulated by asparaginyl hydroxylation, which is mediated by the asparaginyl hydroxylase hypoxia-inducible factor 1-alpha inhibitor (FIH-1) when blood oxygen levels are correct (74, 85). Indeed, FIH-1 mediates hydroxylation of an asparagine residue within the HIFα TAD, which prevents the binding of coactivators such as CBP/p300 to HIFα, thereby reducing HIF-dependent transcriptional activation. However, it is important to note that FIH-1 has higher specificity towards HIF-1α than

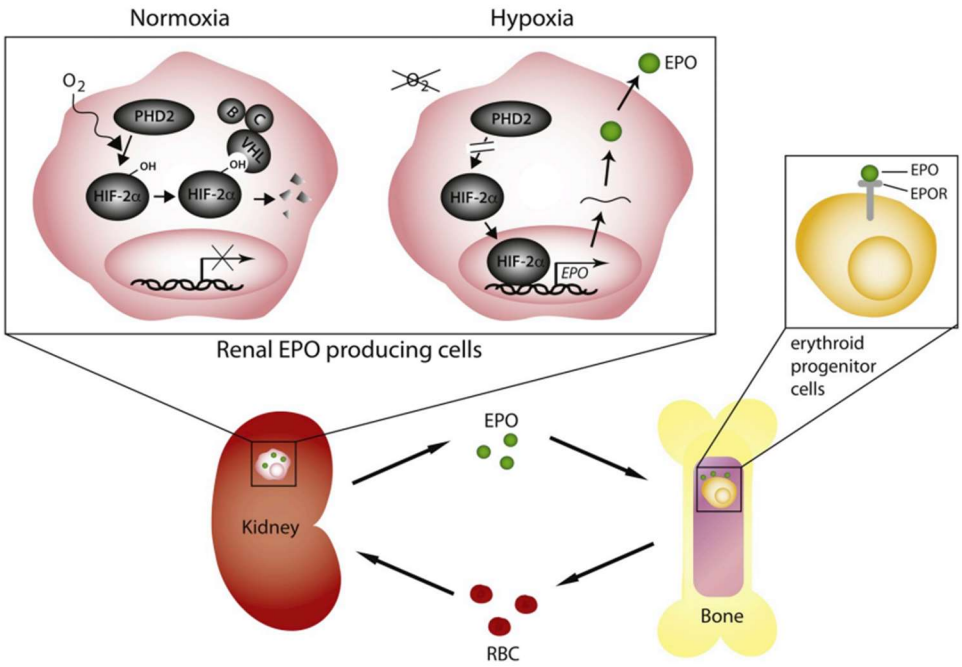


***Figure 4:* The feedback loop that controls red blood cell production**. *The oxygen-sensing mechanism that regulates EPO gene expression in the kidney is illustrated in the upper left panel. Under normoxic conditions, PHD2 hydroxylates one or both of a pair of prolines of HIF-2α in an oxygen-dependent manner, using 2-oxoglutarate and ascorbate as cofactors. This hydroxylation allows for specific binding by an ubiquitin ligase complex containing VHL, elongin B (B), and elongin C (C) that leads to ubiquitination and subsequent proteasomal degradation. Under hypoxic conditions, hydroxylation of HIF-2α is attenuated, allowing it to bind to HRE in the 5′ region of the EPO gene. This engenders the transcription of EPO. EPO enters the circulation and binds to EPO receptors on erythroid progenitor cells (primarily CFU-erythroid cells), which induces proliferation and differentiation of erythroid cells (upper right panel). The mass of circulating red blood cells thus depends on the degree of hypoxia detected by PHD2 in the kidney and reflects the dynamic balance between EPO-induced red blood cell production and subsequent loss or destruction of mature red blood cells. Figure and legend from Lappin et al (86).*

PHD2 (87). Therefore, PHD2 remains the primary oxygen sensor in controlling EPO production, while FIH-1 plays a more selective role in regulating HIF-1α-dependent gene expression (88). Other molecular mechanisms of the HIF-EPO pathway may modulate the stability or activity of HIF-2α and its partners in correlation with erythropoiesis (89).

Recent research indicates that Notch signaling is active in early hematopoiesis (90, 91). The role of Notch signaling in erythropoiesis varies depending on whether it is induced or inhibited. Induction of Jag1-Notch1 signaling promotes the maintenance and expansion of hematopoietic progenitors (92), while inhibition of Notch signaling results in a higher rate of differentiation at the expense of HSC maintenance (93). Oh *et al.* have uncovered a differential action between Notch receptors, with Notch1 being associated with the lymphoid lineage and Notch2 involved in early erythroid differentiation (94). Their investigations confirm the importance of Notch in stress erythropoiesis, indicate that Notch regulation of erythropoiesis is specific to adult hematopoiesis and provide insights for future therapeutic interventions (e.g., using specific Notch 2 ligands to enhance erythropoiesis). Moreover, low oxygen concentration activates Notch signaling and

stabilizes HIFs. Indeed, Labat et al. demonstrated that HIFs, particularly HIF-2, play a crucial role in early erythroid development, in cooperation with the Notch pathway (95). Inhibition of the Notch pathway slightly decreased erythroid cell expansion and promoted their differentiation. HIFs also contribute to the expression of target genes of the Notch pathway, thus influencing the commitment and amplification of erythroid precursors. In HIF-PHD-2 deficient mice, enhanced erythroid growth has been observed with age, primarily in the spleen (96). Hence, stabilization of HIF-1α and HIF-2α in the spleen induced splenic erythropoiesis independently of EPO through downregulation of genes in the Notch pathway (96).

GATA-2 and nuclear factor κB (NF-κB) are other transcription factors that can regulate EPO gene expression. However, unlike HIFα, they have an inhibitory effect on EPO transcription. GATA-2 and NF-κB (activated by proinflammatory cytokines such as IL-1 and TNF-α) can bind to the EPO gene promoter and inhibit its expression (3).

#### *1.1.3.3 EPO receptor*

The erythropoietin receptor (EPO-R) is a member of the class I cytokine receptor superfamily **(Figure 5).** It contains a WSXWS motif in the extracellular domain, a single transmembrane domain, and a cytoplasmic domain with tyrosine kinase activity (97-99). The EPO-R associates with Janus kinase (JAK) and forms homodimeric, heterodimeric or heterotrimeric complexes (98, 100). The plasma half-life of EPO is approximately 8 hours (19) before binding to the EPO-R. This binding of EPO to the extracellular portion of its receptor induces a conformational change bringing the two cytoplasm-associated Janus kinase 2 (JAK2) homodimers together, allowing transphosphorylation of JAK, phosphorylation of the receptor, phosphorylation and activation of the signal transducer and activator of transcription (STAT) and other downstream signaling pathways including phosphatidylinositol 3-kinase (PI3K)/AKT kinase (AKT is also known as protein kinase B) and extracellular signal-regulated kinases (ERK) 1/2 (101, 102). The phosphorylated subunits of STAT5, STAT5A and STAT5B, dimerize and move to the nucleus to activate the expression of certain genes (103). Indeed, STAT5 has regulatory activity on several cell proliferation and differentiation genes and can promote anti-apoptotic genes in erythroid precursors (104).

Interestingly, the EPO-R can be activated by tyrosine phosphorylation by SCF (18). Recently, another pathway involving serpin and lysosomal cathepsin has been described in Ebs with a modulatory action on survival (105). Furthermore, as cited above, the EPO-R induces suppression of Fas/FasL in early Ebs (28, 34).

Many publications highlight the importance of the transcription factor GATA-1 in normal erythropoiesis (23). Indeed, in the absence of GATA-1, committed erythroid precursors fail to mature properly and instead undergo apoptosis. GATA-1 upregulates the expression of erythroid-specific genes, including the gene for EPO-R (106). Moreover, Grégory et al demonstrated that EPO acts in conjunction with GATA-1 to stimulate the expression of the bcl-xL gene. Therefore, EPO and GATA-1 cooperate to maintain the viability of erythroid cells during their late stages of maturation (24). In fact, GATA-1 can bind to EPO-R and enhances the STAT-5 signaling pathway. This up-regulation of the pathway leads to an increase in Bcl-xL expression, thus protecting early erythroid progenitors from cell death mediated by caspase-3 (an enzyme involved in the apoptosis process).

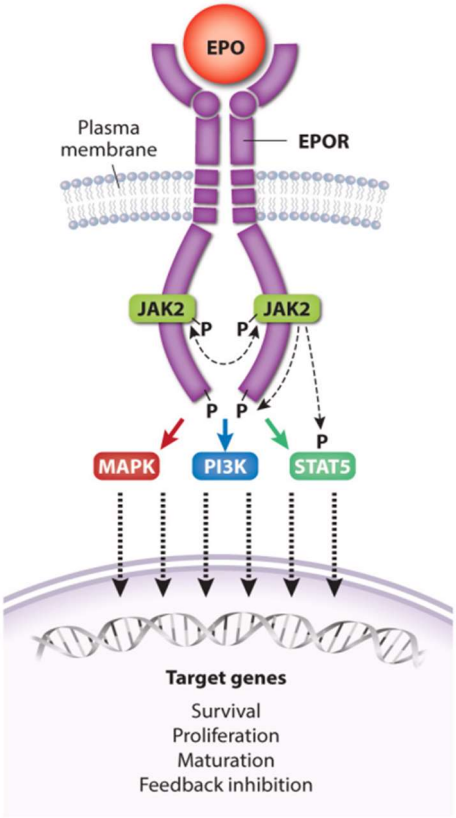


***Figure 5:* Activation of the erythropoietin receptor (EPOR) by EPO.** *A single EPO molecule binds and stabilizes EPOR–JAK2 complex dimers, inducing a conformational change that initiates JAK2 transphosphorylation and activation. Active JAK2 phosphorylates multiple tyrosine residues on STAT5 and the cytoplasmic domain of EPOR, triggering a signaling cascade that activates numerous effector pathways contributing to biological activity. Dashed lines represent kinase activity. For simplicity, the kinase activity of only one JAK2 protein is indicated. Major signaling pathways activated by EPOR include Ras/MAPK, STAT5, and PI3K/AKT, which drive the expression of genes that promote erythroid progenitor survival, proliferation, and differentiation as well as feedback inhibition of EPOR signaling. MAPK, mitogen-activated protein kinase; PI3K, phosphoinositide-3 kinase; STAT5, signal transducer and activator of transcription 5. Figure and legend from Bhoopalan et al (100).*

These results suggest that GATA-1 is crucial for the maturation and survival of erythroid cells, and that its interaction with EPO and the STAT-5 pathway plays an important role in maintaining the viability of these cells during the terminal stages of their development. Furthermore, this focuses a hierarchical relationship in which bcl-xL acts as a crucial downstream effector of GATA-1 and EPO-mediated signals. Note that the activation of STAT5 and Bcl-xL depends on variations in EPO levels, rather than on its absolute values.

#### *1.1.3.4 EPO function*

EPO is essential for marrow erythropoiesis. However, in addition to its primary role in erythropoiesis, the EPO receptor is also expressed on the surface of other cells such as muscle cells, neurons, and immune cells. Subsequently, a pleiotropic role of the EPO response has been demonstrated that may contribute to various physiological manifestations associated with anemia, ischemia or pharmacological uses of EPO.

*Erythropoiesis:* Basal erythropoiesis may increase under hypoxic stress when oxygen supply is acutely decreased (34, 107). In these situations, oxygen supply to the kidneys is reduced, which activates the HIF pathway and increases EPO transcription, thereby raising circulating EPO levels (62, 108, 109). EPO promotes the expansion of BFU-E erythroid precursors and BFU-E precursors, acting through EPO surface receptors that in turn activate signaling pathways such as those involving STAT5, MAPK and/or PI3K/AKT via JAK2 (1, 110, 111).

In addition, Casimir *et al.* recently demonstrated, both in critically ill patients and in a murine model, a physiological role of EPO in downregulating post-transfusion clearance of RBCs, thus contributing to maintaining a vital RBC biomass to rapidly cope with hypoxemia (112). Indeed, severe anemia has been associated with a higher-than-expected increase in Hb after transfusion (113, 114). By observing greater post-transfusion Hb increment in anemic mice exposed to hypoxia or receiving an erythropoiesis-stimulating agent (ESA), smaller increases in mice depleted of macrophages or receiving EPO-neutralizing antibodies or irradiation, and no effect of splenectomy, they suggested a mechanism involving both erythroid progenitors and macrophages, but not specific to the spleen (112).

Other pleiotropic effects of EPO include (115):

- *Bone remodeling* by activation of the osteoblast bone-forming activity.
- *Tissue protection*: EPO has been shown to have a protective effect on various tissues including the brain (neuroprotection), heart, kidney, and liver. EPO can reduce tissue damage and promote tissue repair following injury or ischemia (reduced blood flow). By protecting nerve cells from damage and degeneration, EPO can improve outcomes in conditions such as stroke, traumatic brain injury, and multiple sclerosis.

- *Angiogenesis*: EPO can stimulate the growth of new blood vessels, a process known as angiogenesis. This effect is important for tissue repair and regeneration.
- *Anti-inflammatory effects:* EPO has anti-inflammatory properties and can reduce inflammation in various tissues. This effect is important for reducing tissue damage and promoting tissue repair.
- *Immunomodulatory effects:* EPO can modulate the immune system and enhance the body's ability to fight infections.

Overall, the pleiotropic effects of EPO are believed to be mediated through a variety of mechanisms including the activation of specific receptors on target tissues, the modulation of gene expression, and the regulation of signaling pathways involved in tissue repair and regeneration.

#### *1.1.3.5 Erythropoiesis-stimulating agents*

The discovery of the human erythropoietin gene led to the development of recombinant human erythropoietin (rHuEPO) in the early 1980s, in 2 forms, alpha and beta, which differ in their amino acid sequence (59). Then came a synthetic analog called darbepoetin alpha (DA) and a pegylated form of EPO-beta, which have a longer duration of action (116-118). RHuEPO, DA and pegylated EPO are currently used as erythropoiesis-stimulating agents (ESAs). A comprehensive meta-analysis of 27 studies with 5,410 patients assessed the effect of pegylated EPO compared to other EPO (darbepoetin alfa or EPO alfa/beta) in patients with chronic kidney disease, demonstrating similar efficacy and side effects in terms of mortality, major cardiovascular events, hypertension and transfusion needs while requiring only one injection per month (119). In addition to ESAs, prolyl hydroxylase (PHD) inhibitors and luspatercept are expanding our arsenal in the fight against anemia (see below).

ESAs offer an alternative to transfusion and have been used for over 35 years to improve anemia in patients in a variety of pathological situations. RHuEPO was initially developed to treat chronic renal failure patients suffering from anemia (120, 121). In these patients, endogenous EPO production is reduced, leading to severe anemia. The use of recombinant EPO has made it possible to compensate for this deficiency and to increase RBC production, thereby improving anemia and quality of life in patients achieving RBC transfusion independence (122). Since its introduction in the treatment of anemia in kidney failure, the use of ESAs has spread to other clinical areas such as the treatment of cancer-related anemia (123), in hemoglobinopathies (124), in myelodysplastic syndromes (MDS)(125), after hematopoietic stem cell transplantation (HSCT) (126), in acquired immune deficiency syndrome (127) and in a surgical setting with or without an autotransfusion program (128).

***Erythropoiesis-stimulating agents in the anemia of chronic disease***

The Anemia of Chronic Diseases (ACD) result from persistent inflammatory disorders (129), such as infections, solid cancers and hematological malignancies (130, 131), autoimmune and inflammatory diseases (132), but also kidney and heart failure (133).

The chronic inflammatory process leads to hepcidin secretion via the IL-6/STAT3 pathway (produced by macrophages) resulting in inadequate iron supply to the BM (see 1.2.2.2 Regulation of hepcidin by inflammation and 1.2.3.1 Iron deficiency) (134, 135). In addition, overproduction of inflammatory cytokines directly inhibits erythropoiesis, interferes with renal EPO production in response to anemia, and shortens RBC lifespan (136).

Many cancer patients present with anemia, the cause of which is multifactorial (137) but the main mechanism is ACD (123, 138). Decreased EPO production has been reported in multiple myeloma (139), lymphoma or chronic lymphocytic leukemia (140), and in solid tumors (with or without chemotherapy) (141).

Although the main treatment for ACD is that of the underlying disease, RBC transfusions are often necessary. The use of rHuEPO, which stimulates endogenous erythropoiesis, has been tested in various pathologies and has proved effective (142) for example in cancer with or without chemotherapy (143), in lymphoma and in myeloma (144). However, in some cases, anemia may be caused by complex inflammatory mechanisms, and EPO alone may not be sufficient to fully restore erythropoiesis. Indeed, around 30% of patients with solid cancer or hematological malignancies fail to respond to ESA therapy (143, 145, 146). The main reasons for resistance to treatment is inflammation and absolute or functional iron deficiency (147). This is why it has been shown that treatment of iron deficiency (by intravenous iron injection) should be carried out before giving ESAs, as this improves the erythropoietic response, reduces its use and thereby economic costs (131, 148, 149).

Several meta-analyses on outcomes in anemic cancer patients treated with ESA have been published, but the largest have been the ones carried out by the Cochrane Collaboration, both as a meta-analysis of individual data from 13,933 patients in 53 trials (150) and a later update including 91 trials with 20,102 participants (151). ESAs significantly generate Hb responses and reduce the need for RBC transfusions by 30-40% and the average number of RBC units transfused by one unit. The evidence that ESAs improve quality of life (QOL) is less strong, reaching statistical significance for all assessed QOL tools but clinical significance for only some of them. However, there is also strong evidence that ESAs increase on-study mortality (HR 1.17 [1.06-1.29]) and some evidence that ESAs reduce overall survival (OS, HR 1.05 [1.00-1.11]), whereas an effect on tumor response has not been evidenced. The effects on mortality and OS are not significant in patients with hematological malignancies, those with baseline Hb <12 gr/dL and those receiving chemotherapy with or without radiotherapy. Indeed in lymphoproliferative diseases, ESAs during

chemotherapy do not impact mortality or disease progression (152). In addition, there is strong evidence that ESAs increase the risk for thromboembolic events (TEE, HR 1.52 [1.34-1.74]), essentially in solid tumors and under cancer therapy) and some evidence that they increase the risk of hypertension (HR 1.30 [1.08-1.56], probably an overestimate) and thrombocytopenia (HR 1.21 [1.04-1.42], clinical relevance unknown). There has been a suggestion that the increased risk of TEE correlated with Hb values (153), but this has not been confirmed (154).

***New classes of erythropoiesis-stimulating agents***

Hypoxia inducive factor-prolyl hydroxylase domain (HIF-PHD) inhibitors, i.e. Roxadustat, Vadadustat, Daprodustat, and Molidustat, are orally-administered innovative drugs that inhibit the action of HIF-PHD enzymes, leading to the intracellular accumulation of HIF and subsequent activation of erythropoiesis-related genes, creating a temporary "pseudo-hypoxic" state mimicking the cellular hypoxic conditions responsible for EPO production (155). In addition, PHD inhibitors enhance iron availability for erythropoiesis by reducing hepcidin levels, effectively counteracting functional iron deficiency often observed in case of resistance to classical ESAs (156, 157). Indeed, studies in chronic kidney disease have demonstrated similar efficacy with a reduced need for intravenous (IV) iron supplementation with Roxadustat compared to ESA (157-159).

In solid tumors, the proliferation of cancer cells often outpaces the growth of functional vessels, leading to intra-tumoral hypoxia. In response to such hypoxia, HIFs increase the expression of numerous proteins that further stimulate the growth, differentiation, and dissemination of tumor cells. Available data show that the HIF-PDD inhibitors Roxadustat and Vadadustat do not have tumor-promoting effects in vivo, while Daprodustat, inhibiting PHD1 and PHD3, and Molidustat, inhibiting PHD2, reduce tumor growth and enhance vascular maturation (160), resulting in significant anticancer effects in vivo (161). In addition, a phase 3 study of Roxadustat in patients with low-risk MDS yielded promising results in reducing transfusions (162).

Luspatercept (Reblozyl®) is a first-in-class erythroid maturation agent approved for the treatment of transfusion-dependent anemia in adults with low-risk MDS with ring sideroblasts who have not responded to prior ESAs or for β-thalassemia, diseases characterized by overactivation of the SMAD2 signaling pathway (163, 164) as well as ineffective blood cell production and necessitating numerous RBC transfusions (165, 166). Indeed, overactivation of p38 MAPK in bone marrow progenitors has been described in low-risk MDS patients (167). Inhibiting the p38 MAPK signaling pathway partially rescues hematopoiesis in MDS progenitors (26). Zou *et al.* demonstrated that SMAD2, a downstream mediator of TGF-β receptor I kinase activation, is constitutively activated in bone marrow precursors of MDS and is overexpressed in MDS CD34+ cells, providing direct evidence of TGF-β pathway overactivation in this disease (164).

The ligands within the TGFβ superfamily, including activins, growth differentiation factors, and BMPs, initiate the formation of activated complexes in the SMAD pathway containing various combinations of type I and type II receptors that participate in erythropoiesis regulation (168). Suppression of TGF-β signaling by a luspatercept analog (ACE-536) inhibits such SMAD2 overactivation and leads to enhanced hematopoiesis both in vitro and in vivo in a murine model of bone marrow insufficiency induced by constitutive hepatic expression of TGF-β1 (164). Luspatercept is a recombinant fusion protein consisting of the extracellular domain of ActRIIA linked to the human immunoglobulin G1 Fc domain. It specifically binds and inhibits ligands of the TGF-β superfamily, particularly activin A, thereby reducing SMAD2/3 signaling and promoting the maturation of RBC precursors (166). Its efficacy has been demonstrated in healthy postmenopausal women (169), as well as in low-risk MDS patients resistant to ESAs (170) or in myelofibrosis (171) where inhibition of TGF-β signaling may halt fibrosis and reactivate normal hematopoiesis (172).

In β-thalassemia, uneven production of the α and β globin chains triggers apoptosis and inhibition of late-stage erythroid differentiation, resulting in ineffective erythropoiesis, anemia and dysregulated iron homeostasis, with subsequent transfusion dependence (173, 174). While clinical experience with rHuEPO is limited (174), a murine analog of luspatercept reduced oxidative stress and improved anemia in a murine model of β-thalassemia (175, 176). Subsequently, multiple studies have demonstrated the efficacy and safety of luspatercept, with hemoglobin improvement and reduction of transfusion needs in a significant proportion of β-thalassemia patients (173, 177, 178).

## 1.2 Iron

### 1.2.1 Iron metabolism

#### *1.2.1.1 Role of iron*

Iron is the most abundant element by mass in the Earth (179). Due to its exclusive oxidation-reduction properties, iron readily donates and accepts electrons and exists mainly in the ferric (Fe3+; insoluble form) and ferrous (Fe2+; soluble form) oxidation states (180). This capability makes it a useful component of cytochromes, oxygen-binding molecules (i.e., Hb and myoglobin), and many enzymes.

Iron is an essential element for the fundamental biological mechanisms of nearly all known life forms. It is involved in erythropoiesis, oxygen transport, regulation of the cell cycle, cellular respiration and energy metabolism (181). The human body contains between 3 to 5 grams of iron (35 to 45 mg of iron per kilogram of body weight). Two-thirds of this amount (about 1800 mg of the total body iron) is found in the heme of Hb in erythroid precursors (inside the BM) and mature RBC. Heme is also found in myoglobin in the muscles that contain about 300 mg of iron. Iron is

also stored in the form of ferritin inside hepatocytes (1000 mg) and macrophages (600 mg) in the liver and spleen. More than 95% of iron in plasma is bound to its circulating transport protein transferrin (Tf; 3 mg in total) **(Figure 6)**.

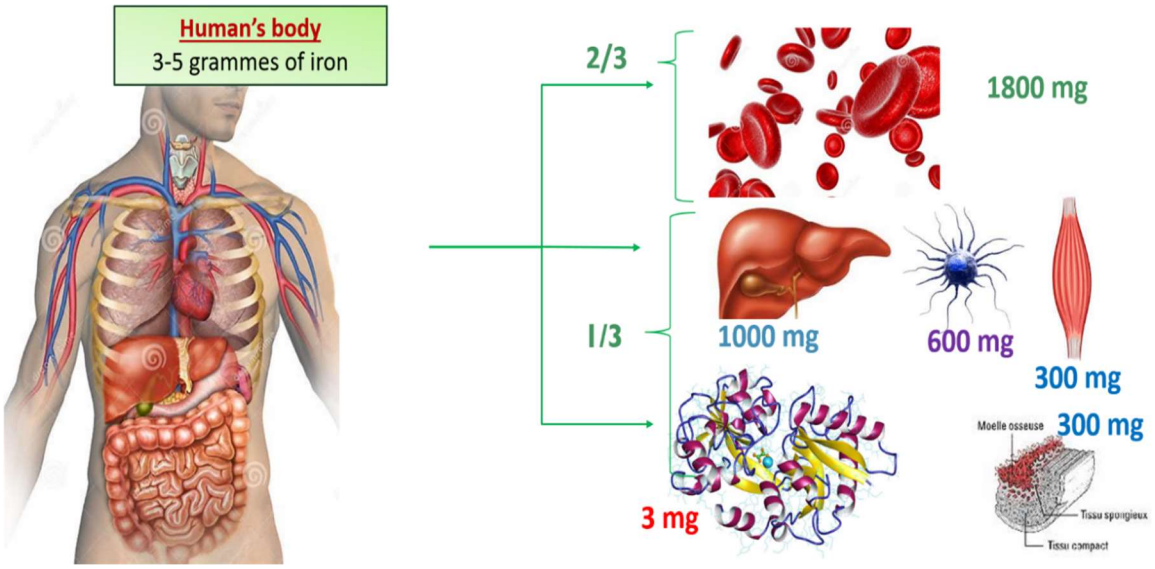


*Figure 6: Distribution of iron in the human body.*

In the BM, most of the heme is incorporated into Hb to transport oxygen in the body, while in the liver, iron is mainly used to produce cytochromes P450 (about 2/3 of hepatic heme) (182). Cytochromes P450 are important enzymes involved in the metabolism of, among others, drugs, steroids, fat-soluble vitamins, and carcinogenic compounds (183).

Iron plays an important role in cell proliferation. Indeed, iron-containing proteins catalyze key reactions implicated in folate metabolism (184) and DNA synthesis (iron is a cofactor of the ribonucleotide reductase for DNA synthesis (185)). In fact, without Fe, Cdk-cyclin complexes (cyclin A, E, Cdc2, Cdk2) are inhibited, which leads to an arrest of the cell cycle in G1 and S phases (the S phase corresponding to the DNA-replication phase). In addition, during DNA replication and repair, activation of iron-sulfur (Fe-S) cluster-containing proteins, such as DNA polymerases and DNA helicases, requires prosthetic fragments (186). Iron is as well implicated in mitochondrial processes used in cellular respiration. It serves as a cofactor for many proteins of the respiratory chain, such as proteins containing Fe-S iron-sulfur clusters (NADH dehydrogenase, subunits of succinate dehydrogenase), hemoproteins (cytochromes, succinate dehydrogenase), and proteins containing iron-ion groups, allowing electron transfer. The latter leads to the

production of ATP (adenosine triphosphate) which provides the energy necessary for the chemical reactions of the organism (187).

Iron is also involved in cell death through ferroptosis, a concept proposed by Dixon et al in 2012, which continues to be studied in different pathological situations (188). It is a non-apoptotic, iron-dependent, programmed cell death characterized by an accumulation of reactive oxygen species (ROS, Reactive Oxygen Species) derived from iron metabolism, inducing lipid peroxidation. Excessive intracellular iron contributes to ferroptosis by producing ROS through the Fenton reaction. Morphologically, ferroptosis is characterized by the presence of smaller than normal mitochondria with condensed density of mitochondrial membranes, reduction or disappearance of mitochondrial cristae, and rupture of the outer mitochondrial membrane. However, the cell membrane remains intact, the nucleus is of normal size, and there is no chromatin condensation distinct from other types of cell death (189). Decreasing intracellular iron overload (IO) by iron chelation inhibits chemically induced ferroptosis, whereas exogenous
iron supply stimulates it (190). Ferroptosis is involved in many physiological and pathological processes, such as cancer cell death, neurotoxicity, neurodegenerative diseases, acute renal failure, and liver toxicity (191-195).

Under important physiologic states, in conditions of IO, excess iron catalyzes the formation of free radical ions via the Fenton reaction ($Fe^{2+} + HO^- \rightarrow Fe^{3+} + OH^- + OH$) that may be harmful to cells (196). The formation of hydroxyl radicals leads to significant cellular damage by inducing oxidative stress and thus oxidizing biomolecules such as DNA – by altering their electrical charge and causing cross-links between proteins – and lipids – by inducing lipid peroxidation and disorganization of the phospholipidic bilayer (197).

Besides, iron is also necessary for the growth of pathogens (bacteria, fungi…) (198). Consequently, binding of iron to proteins such as ferritin, Tf and lactoferrin helps to create a low iron environment in the body, limiting the availability of iron to pathogens and regulating its exchange to maintain optimal iron homeostasis.

#### *1.2.1.2 Body iron exchange*

Dietary iron is the only source of iron in the body (about 10 to 20 mg of iron per food bolus/day). One to two milligrams of iron from the food bolus are absorbed each day in the gut (199). This intake only compensates for the daily loss of iron due to bleeding, desquamation of intestinal and urinary tract cells, and skin cells (200). However, the human body needs much more iron, in particular for erythropoiesis, which requires twenty to twenty-five milligrams of iron each day to produce two hundred billion RBC (201). As the dietary intake is largely insufficient, this demand is covered by recycling iron from senescent or damaged RBC (201). To achieve this recycling, macrophages, the body's "lumpy" cells, capture RBC at the end of their life and recover the iron by

digesting their Hb. This process, called "*erythrophagocytosis",* recycles iron to make it available to the BM for the synthesis of new RBC (202) **(Figure 7).** In addition to this process, haptoglobin and hemopexin scavenge and transport heme-derived molecules for cellular clearance. However, pathological hemolysis may exceed the capacity of macrophages and these scavengers across a variety of diseases (203).

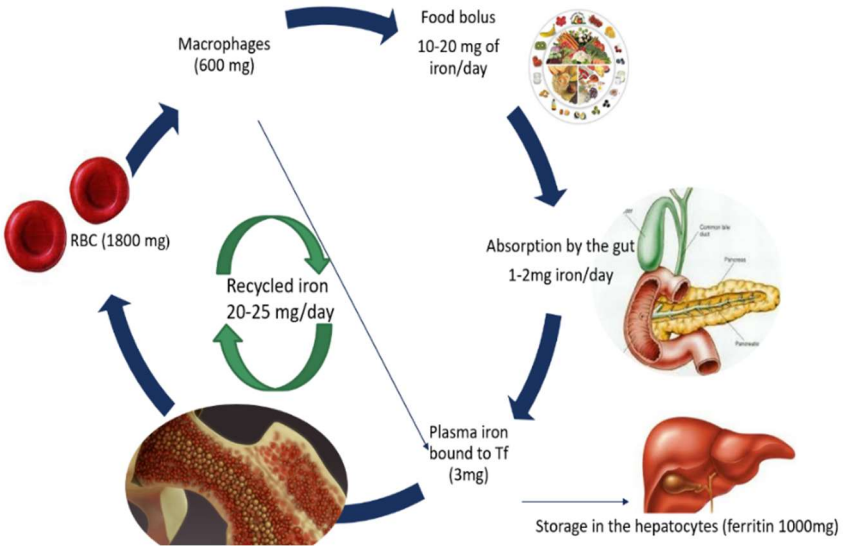


***Figure 7*: Body iron exchange.** *Tf: transferrin; RBC: red blood cells.*

Duodenal enterocytes are polarized cells with an apical pole in contact with the intestinal lumen and food bolus, and a basolateral pole oriented towards the bloodstream. The apical pole has a brush border containing microvilli that increase the surface area for assimilation of nutrients in the diet. Iron is absorbed by different mechanisms depending on its form **(Figure 8).** The iron contained in food exists in two forms, heme iron (mainly from animal sources) and non-heme iron (mainly from plant sources).

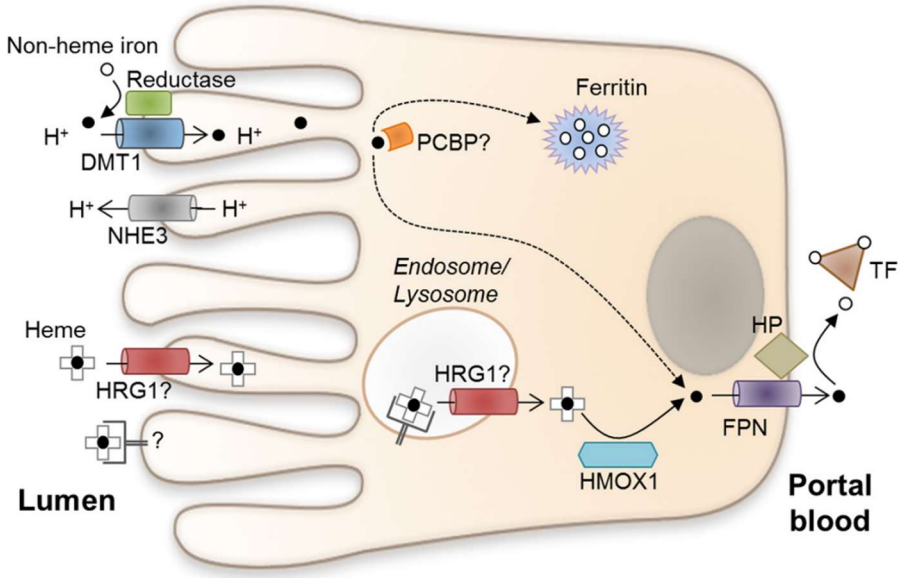


***Figure 8*: Iron transporters in the enterocyte.** *Dietary non-heme iron in the acidic microclimate at the absorptive surface of the proximal small intestine is reduced to Fe2+ by DCYTB and transported across the apical membrane of enterocytes via DMT1. This reduction produces H+ ions which are transported by Na+/H+ exchanger 3 (NHE3) outside the enterocyte. Heme enters through HCP1 (regulated by heme responsive gene, HRG), is then catabolized by heme oxygenase 1 (HMOX1) in the enterocyte. Next, intracellular Fe2+ can be incorporated into ferritin, possibly through members of the poly(rC)-binding protein (PCBP) family of metallochaperones. Alternatively, Fe2+ can be exported into the bloodstream by ferroportin (FPN). This export is followed by oxidation by hephaestine (HP). The oxidized iron Fe3+ is then transported by transferrin (TF) to the cells expressing transferrin receptors. DMT1: divalent metal-ion transporter-1; NHE3: Na+/H+ exchanger 3; HRG1: heme-responsive gene-1; PCBP: poly(rC)-binding protein; FPN: ferroportin; HMOX1: heme oxygenase 1; HP: hephaestin; TF: transferrin. Figure from Knutson et al (204).*

### *Absorption of non-heme iron*

Non-heme iron present in the intestinal lumen is in the form of ferric iron (Fe3+; the main form of iron in food) and is converted into ferrous iron (Fe2+) by the ferroreductase DCYTB (Duodenal CYTochrome B) present at the apical pole of enterocytes. However, Gunshin *et al.* have shown that deletion of DCYTB in vivo (Cybrd1-null mice) causes little or no apparent defects in iron metabolism even in the context of iron deficiency (205). This suggests that DCYTB can be dispensable for iron absorption or that other ferroreductases exist. Ferrous iron (Fe2+) is then transported across the apical membrane of duodenal enterocytes via divalent metal transporter 1 (DMT1), but also in endosomes to allow iron efflux into the cytosol (206). DMT1 is a transporter also present on the surface of hepatocytes, macrophages and in the placenta (207).

The importance of DMT1 in iron homeostasis has been demonstrated in mice with microcytic anemia and in Belgrade rats that exhibit systemic iron deficiency and anemia (208, 209). The underlying cause of this condition in both models can be attributed to the same spontaneous missense mutation (G185R) in the solute carrier family 11 member (2Slc11a2) gene encoding DMT1. The G185R missense mutation in Slc11a2 affects the function of the protein, resulting in impaired iron transport. As a result, affected animals are unable to effectively absorb iron from

their diet and to transport serum iron (SeFe) into erythroblasts, resulting in systemic iron deficiency and microcytic anemia.

Another interesting fact is the case of a patient with a different missense mutation in the SLC11A2 gene causing anemia but with hepatic IO (210). This results in incorrect splicing with two types of mRNA molecules produced. The correctly spliced mRNA encodes a variant of the DMT1 that retains its iron transport capacity at the gut and liver level. However, the poorly spliced mRNA encodes a protein that lacks one of its transmembrane segments, rendering it unable to efficiently transport iron into erythroblasts. Indeed, DMT1 is essential for intestinal absorption of non-heme iron but also to produce Hb in erythroid precursors during erythropoiesis (207).

***Absorption of heme iron***

Heme iron is mainly present in the hemoproteins of meat, such as Hb and myoglobin. Heme is released thanks to the combination of the low pH of the stomach and the proteolytic activities of enzymes. The Heme Carrier Protein 1 (HCP1) transporter, present at the apical pole of enterocytes, has been identified as a possible heme transporter, although it is more affine for folate transport (211, 212). Heme oxygenase-1 (HMOX1), present in the cytoplasm, then degrades heme, releasing iron into the cell (213). Heme iron could also be taken up by enterocytes through endocytosis, that occurs by receptor -mediated endocytosis (RME) present on the plasma membrane (214). Heme oxygenase-2 (HMOX2) is another isoform of the enzyme. While HO2 is primarily found in the cytoplasm, there is evidence suggesting its presence in endosomes from HO2/endosome co-labeling experiments (214). On the other hand, DMT1 is not present in endosomes. The transporter involved in exporting iron from lysosomes/endosomes is Heme Responsive Gene 1 (HRG1), also known as Feline Leukemia Virus Subgroup C Receptor 1 (FLVCR1) (215).

Iron is transported out of the enterocyte and into portal blood via ferroportin (SLC40A1) located on the basolateral membrane (216). Nevertheless, part of the iron is stored by enterocytes in the form of ferritin that is lost when enterocytes are shed into the gut lumen. The mechanism by which iron taken up by DMT1 at the apical membrane reaches basolateral ferroportin is not fully understood. However, it is believed that the process involves certain proteins known as poly(rC)-binding proteins (PCBP) that act as metallochaperones. PCBP1 and PCBP2 have been identified as iron chaperones that facilitate the transport of cytosolic iron to ferritin for storage (217).

To date, ferroportin is the only iron exporter identified in mammals. This protein is expressed mainly on the basolateral surface of polarized epithelial cells, such as enterocytes (218), as well as on the surface of macrophages in the spleen, Kupffer cells in the liver, kidney and heart (219) Mice with intestine-specific deletion of ferroportin accumulate iron in duodenal enterocytes and become severely anemic, showing that this protein is crucial for intestinal iron transfer (220).

Ferroportin transports only Fe2+ whereas plasma Tf will bind only Fe3+ (221). To bind to Tf, the oxidation of Fe2+ to Fe3+ is mandatory and occurs through hephaestin (222). This transmembrane oxidase is predominantly expressed in the villi, but not in the crypts. Hephaestin contains a heme group and a copper atom in its structure. The copper atom is essential for the enzymatic activity, as it acts as a cofactor in the oxidation of Fe2+ to Fe3+. The heme group is believed to serve as an electron carrier in the reaction.

#### *1.2.1.3 Iron utilization and storage*

**Ferritin**

Iron in the enterocyte can either be used for cellular needs, be stored in *ferritin*, or be exported to the bloodstream through ferroportin. When holo-transferrin (Tf bound to iron) interacts with TfR-1 on the surface of cells, it forms the Tf/TfR1 complex, which is then internalized into endosomes. The endosome is an acidic compartment where iron is released from Tf and transported into the cytoplasm (180). However, iron needs to be reduced to Fe2+ before being transported by DMT1 across the endosomal membrane into the cytoplasm. The reduction of Fe3+ to Fe2+ is catalyzed by the six-transmembrane epithelial antigen of the prostate 3 (STEAP3) reductase in the endosomal membrane (223). Once in the cytoplasm, Fe2+ can be further transported into mitochondria to be incorporated into heme or iron-sulfur clusters. The latter are important components of many cellular processes, including electron transport and DNA synthesis. Alternatively, Fe2+ can be incorporated into iron-containing proteins, such as myoglobin and cytochromes, which also play important roles in cellular function **(Figure 9).**

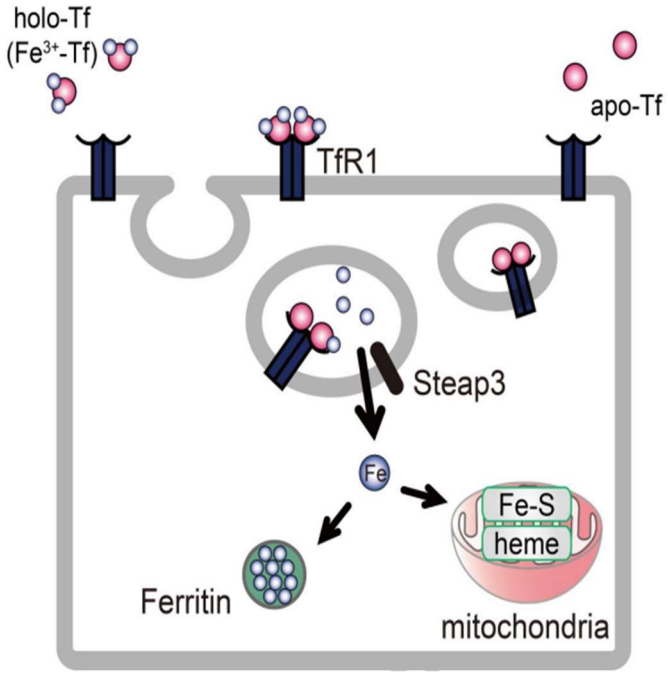


***Figure 9*: Cellular iron metabolism.** *Serum iron is bound to transferrin (holo-transferrin) which interacts with its cell surface receptor TfR-1. The Tf/TfR1 complex is internalized in the endosomes. Iron is reduced to Fe2+ before being transported by DMT1 across the endosomal membrane to be released into the cytoplasm. The reduction of Fe3+ to Fe2+ is catalyzed by six-transmembrane epithelial antigen of prostate 3 (Steap3). Once in the cytoplasm, iron is either stored as ferritin or transported into the mitochondria to be incorporated into heme or other iron-containing proteins (myoglobin). Holo-Tf: holo-transferrin; TfR1: transferrin receptor 1; apo-Tf: apo transferrin; Steap3: six-transmembrane epithelial antigen of prostate 3; Fe: iron; Fe-S: iron-sulfur. Figure from Iwai* et al (224).

However, when there is an excess of iron that cannot be immediately used by the cell, it is stored in the form of ferritin. Ferritin is a ubiquitous protein allowing the storage of 4500 iron atoms in a non-toxic form, which can be degraded to make it bioavailable (225). It serves as a sensor of intracellular iron levels and responds to fluctuations in iron availability. When iron levels are low, ferritin synthesis is repressed to conserve iron, while high iron levels lead to increased ferritin synthesis to store excess iron. Ferritin is composed of 24 subunits, including heavy (H) and light (L) chains (226, 227). The expression of ferritin genes can vary across different tissues and cell types. The H subunit possesses ferroxidase activity, which is responsible for the conversion of Fe2+ (ferrous iron) to Fe3+ (ferric iron) when iron enters the ferritin shell, sequestering iron in a stable and less reactive form to minimize the risk of oxidative damage (228). H chain expression is often higher in the heart. On the other hand, the L subunit facilitates the nucleation of the iron nucleus and its expression is higher in the liver or spleen (228). Together, the H and L subunits work in concert to control the entry, oxidation, and storage of iron within the ferritin protein complex. In general, both ferritin H and L chains are expressed in most cell types, but H/L ratios can vary. In response to pro-inflammatory cytokines, such as interleukin-6 (IL-6) and TNF-α, the abundance of H subunits tends to increase (229). The H subunit has been suggested to have anti-

inflammatory properties by sequestering free iron and reducing iron-mediated oxidative stress. Additionally, it may contribute to modulating immune responses and regulating iron availability during inflammatory processes. In mice, complete invalidation of the ferritin H gene leads to early embryonic lethality (230). The ferritin-H+/- haploinsufficiency model shows alterations in the brain, a halving of the stock of H chain associated with an increase in TfR, L chain subunit, DMT1 and ceruloplasmin, suggesting a state of iron deficiency. These mice would also show oxidative stress in the brain (231).

In addition to its role as a cytosolic intracellular iron storage protein, ferritin can be found associated with various vesicles (lysosomes, endosomes, autophagosomes) within the cell as a reservoir from which iron can be released when cellular iron demand increases (232). Then, cytosolic ferritin can be induced, as a protector, to counteract the effects of oxidative stress or inflammation. Moreover, some cells, such as neuronal cells and pancreatic islet cells, also express a mitochondrial form of ferritin (233). Mitochondrial ferritin plays a role in iron storage and metabolism within the mitochondria, which is important for maintaining mitochondrial function and preventing iron-dependent oxidative damage (234).

Under normal physiological conditions, a small amount of ferritin is present in the serum. Serum ferritin (SF) is mostly composed of light, iron-poor and partly glycosylated L chains, indicating predominantly hepatocyte secretion (235). Other cell types, such as Kupffer cells (236) and alveolar macrophages (237), can also release ferritin. Several secretion pathways have been described. Studies initially demonstrated secretion via the classical reticulum-endoplasmic and Golgi pathways (235). However, the selective autophagy receptor NCOA4 (nuclear receptor coactivator 4) plays a crucial role in the entry of ferritin into lysosomes, bypassing the endoplasmic reticulum (229). NCOA4 recognizes and binds to ferritin, facilitating its delivery to the lysosomes through a process called ferritinophagy (238). Once inside the lysosome, ferritin is degraded, and the iron is released for cellular utilization. This pathway increases when the cell needs iron for its cellular utilization. Conversely, when iron levels are high, the entry of ferritin into the lysosome is decreased (239). Other non-classical, NCO4-independent lysosomal secretion pathways have also recently been described (240).

It has been known for nearly 50 years that SF levels reflect storage iron status (241). By performing phlebotomies in healthy subjects, Walters et al demonstrated a correlation between SF levels and iron stores, where 1 ng/mL ferritin corresponds approximately to 8 mg/kg of storage iron (242). Normal SF levels are between 12-25 and 300 ng/mL, depending on age and sex. In clinical practice, SF can be used to diagnose absolute iron deficiency or IO, except in certain circumstances that will be discussed later.

**Hemosiderin**

Hemosiderin serves as a long-term insoluble form of storage iron that is less bioavailable than ferritin. It is commonly detected in cells of the reticuloendothelial system, including splenic macrophages, Kupffer cells, and BM macrophages. When there is an excess of iron, cellular ferritin becomes saturated and unable to accommodate further iron storage. As a result, it is converted into hemosiderin through ferritinophagy (243) whereby iron is converted to a crystalline form and deposited in complex aggregates along with residual ferritin, proteins, and lipids (244) within intracellular endomembrane structures called siderosomes **(Figure 10)** (245). When reacting with potassium ferrocyanide, hemosiderin produces an intense blue color (Prussian blue), allowing for its identification and localization within tissues (246). Under the microscope, hemosiderin is observed as brown granules located. The current iron mobilization/storage model follows a labile iron pool (LIP)-hemosiderin flux and vice versa. LIP is the fraction of iron that is not tightly bound to proteins or stored in ferritin or hemosiderin and is readily available for cellular processes. Only the LIP to ferritin and hemosiderin to LIP pathways have been confirmed experimentally (243) **(Figure 10)**.

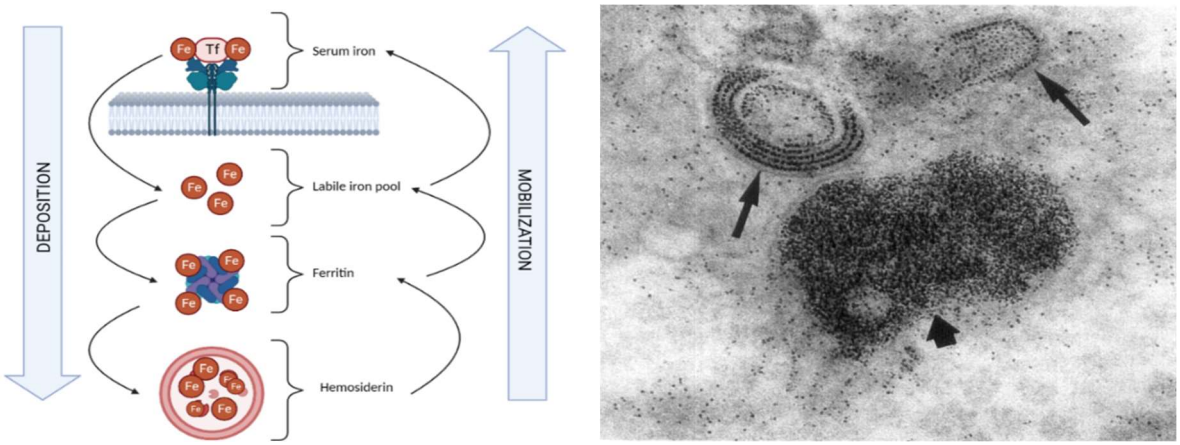


***Figure 10:*** *Left (adapted from Saito et al (243): Iron mobilization/storage model that fits a labile iron pool flux to ferritin or hemosiderin and vice versa. Right: Hepatocyte in electron microscopy (unstained; X 100000) from a child with homozygous thalassemia published by Iancu et al (247): In iron overload, ferritin becomes saturated. Some ferritin particles in the cytosol can then form siderosomes (larger, electron-dense particles contained within an intracellular membrane; arrow). Other particles, unable to form siderosomes, coalesce into agglomerates called hemosiderin (broad arrow).*

## 1.2.2 Iron homeostasis

As described above, iron is essential for many cellular processes, but it can also be toxic if it accumulates to excessive levels. Its cellular metabolism is therefore tightly regulated.

The regulation of iron absorption, storage and utilization in the body is a complex process involving multiple proteins and mechanisms. The fine regulation of iron storage and utilization at the cellular level is achieved by regulating the expression of iron uptake and storage proteins

(TfR1 and ferritin, respectively) according to cellular iron levels. When iron levels are high, cells decrease TfR1 expression to prevent uptake of SeFe and increase ferritin expression to store excess iron. In contrast, when iron levels are low, TfR1 synthesis increases, facilitating iron uptake and ferritin synthesis decreases, enabling storage iron release.

The body must also regulate the entry and recycling of iron at a more global level, involving systemic mechanisms.

### *1.2.2.1 Intracellular regulation*

**Iron Responsive Elements (IRE) – Iron Regulatory Proteins (IRP)**

IRP1 and IRP2 (also known as IRE-Binding Protein 1 and 2) are stem-loop RNA structures present in the untranslated regions (UTR) of certain messenger ribonucleic acids (mRNA) that encode proteins involved in iron metabolism (248). They can bind to specific RNA sequences called iron responsive elements (IRE) that are in the 5'UTR or 3' UTR of mRNAs. Five IREs are in the central part of the 3' UTR region of TfR1 mRNA, whereas one IRE is in the 5'-UTR region of ferritin (L and H) mRNA. No IRE could be found on the mitochondrial ferritin transcript although it is also involved in the regulation of cellular iron metabolism. IREs function as cis-regulatory elements that control the post-transcriptional expression of genes (including tTf, ferritin and DMT1) in response to changes in cellular iron levels (249).

When IRP1 and IRP2 bind to an IRE in the 5' UTR of the ferritin mRNA, they prevent the translation of the mRNA into protein, decreasing ferritin production. In contrast, when IRP1 and IRP2 bind to an IRE in the 3' UTR of the TfR1 and DMT1 mRNA , they stabilize the mRNA and increase its translation into protein **(Figure 11 (224)).**

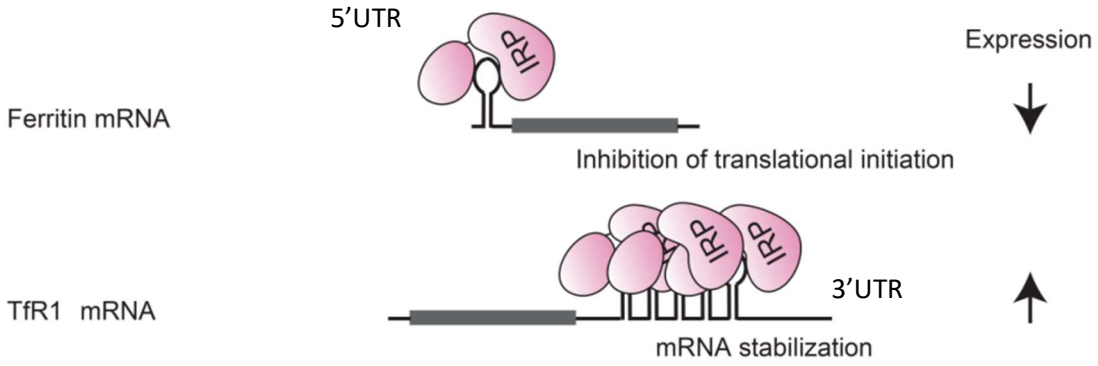


***Figure 11*: Intracellular regulation of iron by the IRE/IRP system.** *When iron requirements increase, iron responsive element protein 1 and 2 (IRP 1 and 2) bind to an iron responsive element (IRE) in the 5' UTR of ferritin mRNA preventing its translation into protein, which decreases ferritin production. In contrast, when IRP1 and IRP2 bind to an IRE in the 3' UTR of TfR1 and DMT1 mRNA, they stabilize the mRNA and enhance its translation into proteins, increasing iron acquisition. In the condition of iron overload or when iron needs are satisfied, IRPs no longer bind to IREs, and the reverse occurs. Figure from Iwai et al (224).*

IRP1 and 2 regulate ferritin and TfR1 gene expression by binding to IRE with high affinity only under iron-depleted conditions. When cells are depleted of iron, IRPs bind to IREs, increasing TfR1 and decreasing ferritin synthesis. This enhances cellular iron supply and consumes iron stored in the form of ferritin. Conversely, under conditions of high iron availability, the ability of IRPs to bind to mRNA is lost when the IRP1 protein incorporates iron atoms into a Fe-S cluster present in its structure. The ubiquitin system is also involved in cellular iron regulation, and the ubiquitin ligase SCFFBXL5 plays a key role in this process (250). When cellular iron levels are normal or high, the substrate recognition subunit of the SCFFBXL5 complex, FBXL5, can sense this change via its hemerythrin-like domain. This leads to the ubiquitination and subsequent degradation of both IRP1 and IRP2. As a result, IRPs no longer bind to the 3'-UTR region of TfR1 mRNA or the 5'-UTR region of ferritin mRNA resulting in decreased expression of TfR1 and increased expression of ferritin. This feedback loop helps maintain iron homeostasis in the cell by regulating the expression of iron-related genes in response to changes in iron availability.

There are other genes transcribed into mRNAs containing IREs. These mRNAs include the erythroid form of aminolevulinate synthase (ALAS2), ferroportin, and HIF, all of which have an IRE in their 5'-UTR (251). Heme synthesis involves the insertion of Fe2+ into protoporphyrin IX, which is synthesized through a series of enzymatic reactions, including the rate-limiting step catalyzed by ALAS2 (erythroid-specific form that is primarily responsible for heme synthesis in RBC). Under conditions of low iron availability, IRPs bind to IREs present in the mRNA of ALAS2, suppressing its translation and thus reducing the rate of protoporphyrin IX synthesis. This mechanism ensures that cells do not waste iron by synthesizing heme when iron supply is limited. Consequently, the IRE/IRP system plays a critical role in regulating heme synthesis in response to changes in cellular iron availability.

HIF-2α is also suppressed by the IRE/IRP system (252). Indeed, Sanchez et al showed that HIF-2α is inhibited in hypoxia and iron deficiency, resulting in decreased production of EPO and thus erythropoiesis. However, as described above, HIF-2α is regulated by its proteasome-mediated and oxygen-dependent degradation. Therefore, the regulation of HIF-2α is very complex, and both iron and oxygen are involved (**Figure 12 (252)).**

Both DMT1 and ferroportin mRNAs contain an IRE that is only present in a subset of their mRNA variants among the multiple splice variants reported. Therefore, the direct involvement of the IRE/IRP system in systemic iron regulation through the regulation of these two genes is still a topic of debate. It is known that the expression of these genes is also regulated by other factors such as HIF and hepcidin. Nonetheless, the presence of the IRE in their mRNA suggests that the IRE/IRP system may have a regulatory role in their expression, especially under conditions of altered iron availability.

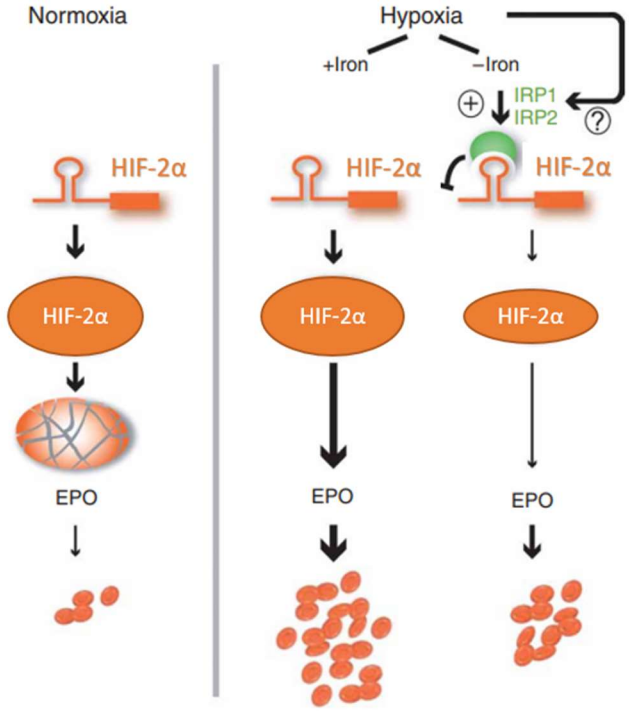


***Figure 12*: Proposed model for the feedback regulation between iron and oxygen metabolism via the IRE-IRP regulatory system and the 5'-UTR IRE of HIF-2α mRNA**. *HIF-2α is degraded by the proteasome in normoxic cells, whereas it is stable during hypoxia, stimulating EPO synthesis and enhancing erythropoiesis. When iron levels are adequate and meet the demand of increased heme synthesis, the mechanism controlling HIF-2α protein stability serves physiologic needs. However, under conditions of iron deficiency, stimulation of erythropoiesis would lead to the accumulation of hypochromic and microcytic red blood cells. To avoid such a situation, EPO stimulation of erythropoiesis should be diminished under low-oxygen conditions when iron is limiting. Sanchez et al propose that this can be achieved by the IRE-IRP system, which would attenuate translation of HIF-2α via its 5'-UTR IRE and allow negative-feedback control when iron is scarce. Question mark at top right denotes that reports on the effect of hypoxia on the IRPs in cultured cells have been contradictory (253). Figure from Sanchez et al (252).*

### Other intracellular regulation systems

Other systems, independent of the IRE-IRP pathway, regulate intracellular iron metabolism.

**The first system** involves the HIF factor, which can regulate the transcription of genes encoding proteins involved in intracellular iron transport. Oxygen transport, erythropoiesis and iron metabolism are strongly linked (53). HIF-1α and HIF-2α are expressed differently: HIF-1α is ubiquitous, while HIF-2α expression is restricted to certain tissues, mainly the duodenum, kidney, and lung. As described in section 1.1.3, HIF-1α is the main regulator of EPO expression. Iron deficiency or anemia can induce tissue hypo-oxygenation, leading to increased expression of HIF-1α. HIFs are sensors of intracellular iron levels but also regulators of iron-related proteins. Indeed, HIF can suppress hepcidin production. The hepcidin-inhibiting effect of HIF was thought to be direct (254) but is now considered as an indirect action via erythroferrone (255). Then, HIF-2α plays a role in regulating intestinal iron absorption. In mice, specific deletion of the HIF-2α gene in enterocytes results in a significant decrease in the expression of DMT1, DCYTB and ferroportin

involved in iron absorption (256, 257). This reduction of iron absorption caused by deletion of the HIF-2α gene in the intestine may even reduce IO in mice lacking the Hamp1 gene (Hamp1-/-) (258). The regulation of DMT1 and DCYTB by HIF-2α was confirmed by the identification of specific HRE sites recognized by HIF-2α in their promoters (257). Overall, deletion of HIF-2α in the gut results in reduced iron absorption and, subsequently, iron deficiency in the body. These observations suggest that HIF-2α enhances iron transport from cells (intestinal and macrophages) to plasma Tf. HIF-1α has also been described to induce heme oxygenase (259), ceruloplasmin (essential for Fe2+ iron oxidation), Tf and TfR1 (260-262), implying that HIF also promotes iron transport in the blood and iron uptake in hematopoietic cells. NCOA4, which is involved as a receptor in ferritinophagy, has also been described to be induced by HIF2α, suggesting that iron uptake from iron stores is also enhanced (263).

**The second system** involves the regulation of intestinal iron absorption by a mechanism called the "mucosal block". The mucosal block refers to a phenomenon whereby the absorption of oral iron is inhibited following the administration of a recent prior dose of oral iron (264). The administration of oral iron increases hepcidin levels (see 1.2.2.2 below), which in turn inhibit the absorption of subsequent iron doses by internalization of ferroportin. However, in mice with intestinal ferritin H deficiency (265), iron absorption is elevated even when hepcidin regulation is intact. These results indicate that hepcidin-mediated regulation alone is insufficient to restrict iron absorption and that intestinal ferritin H is also required to limit iron efflux from intestinal cells (266). By simultaneously deleting IRP1 and IRP2 specifically in mouse enterocytes, Galy et al showed that despite increased expression of both the apical and basolateral iron transporters, derepressed ferritin expression produces a mucosal block by sequestering iron in the cytoplasm (267). Therefore, IRPs appear to ensure adequate iron transport through enterocytes by restricting the ferritin mucosal block and defining baseline iron absorption upon which IRP-independent hepcidin-driven systemic regulation takes place.

#### *1.2.2.2 Hepcidin, master regulator of systemic iron homeostasis*

**Discovery**

Hepcidin is the key regulator of systemic iron metabolism (268). It is mainly produced by hepatocytes and acts as a hyposideremic peptide hormone. Initially, hepcidin was identified as an antimicrobial peptide called liver-expressed antimicrobial peptide 1 (LEAP-1) (269, 270). Human hepcidin is encoded by a single gene called hepcidin anti-microbial peptide gene (HAMP). Interestingly, mice have two genes, Hamp1 and Hamp2. However, the Hamp2 gene has no significant impact on iron metabolism (271). In contrast, the regulation of the mouse Hamp1 gene closely resembles that of the human HAMP gene, making it the closest homolog to the human hepcidin gene (272). The role of hepcidin in the regulation of iron metabolism has been confirmed

by studies using genetically modified mice. Usf2-/- mice, in which the Hamp1 and Hamp2 genes were accidentally inactivated, were the first model to demonstrate IO resulting from hepcidin deficiency (273). To provide further evidence of hepcidin's role, TTR-Hamp1 mice were generated. These mice constitutively overexpress hepcidin in the liver using a specific promoter called transthyretin (TTR) (274), resulting in very low iron stores and anemia. The identification of mutations in the HAMP gene in hemochromatosis patients, resulting in hepcidin deficiency, provided definitive evidence of the crucial role of hepcidin in human iron metabolism (275). Hepcidin-deficient mice develop enormous IO in many organs (liver, pancreas, heart…), which appears early in life (276). The spleen of Hamp1-/- mice is devoid of iron, showing that splenic macrophages are depleted of their iron stores. These different mouse models have established that hepcidin can negatively regulate iron release into the blood in both enterocytes and macrophages.

**Synthesis and excretion**

In humans, the hepcidin peptide is encoded by three small exons within the HAMP gene located on chromosome 19q13.1. The gene encodes a pre-propeptide consisting of 84 amino acids and containing a furin cleavage site responsible for its processing into the mature form of hepcidin (277). Hepcidin is primarily expressed and processed by hepatocytes and is eventually excreted by the kidneys. In the urine, the predominant form of hepcidin is a 25-amino acid peptide known as hep-25 (269). This peptide carries a charge of +2 due to the presence of positively charged amino acid residues. Additionally, two shorter inactive forms of hepcidin, hep-22 and hep-20, have been detected in the urine as well. Although easily crossing the glomerular membrane, the fraction of hepcidin that is finally excreted in the urine is relatively small, suggesting that a significant portion is reabsorbed by the proximal tubule (278). This reabsorption is facilitated by endocytosis, with the megalin receptor playing a key role in this process (279). Megalin is a multiligand receptor expressed on the apical surface of the proximal tubule cells to internalize various molecules, including proteins and peptides, from the tubular fluid.

**Function**

Once in the bloodstream, hepcidin plays a crucial role in regulating iron metabolism. It acts by inducing the internalization of ferroportin, the sole known iron exporter in mammals (216), leading to its degradation in the lysosome (280). Ferroportin consists of 12 transmembrane domains and is expressed in various cell types, including duodenal enterocytes, hepatocytes, and macrophages (281, 282). Upon binding to ferroportin, hepcidin induces the ubiquitination of specific lysine residues, primarily lysine 240 and 258 (283). This ubiquitination leads to the internalization of ferroportin through endocytosis and its subsequent degradation. When

ferroportin is degraded, iron is trapped inside the cell, reducing plasma iron concentration. Aschemeyer *et al.* shed light on another mechanism independent of lysosomal degradation, whereby hepcidin binding in the main cavity of the ferroportin protein leads to occlusion of the cavity, thereby inhibiting iron export through ferroportin (284).

When the iron load is high, hepcidin production increases and induces internalization of ferroportin, leading to reduced iron absorption by the gut and reduced iron release by macrophages, thus helping to prevent IO. Conversely, when iron levels are low, hepcidin production decreases, encouraging increased absorption and release of storage iron to meet the body's iron requirements **(Figure 13).**

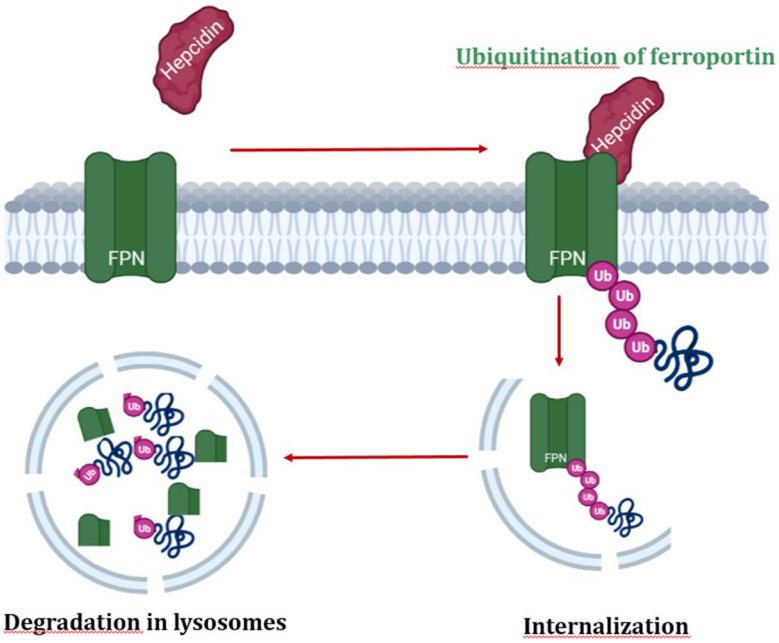


***Figure 13: Regulation of ferroportin by hepcidin.*** *Hepcidin binds to the transmembrane protein ferroportin on the cell surface of enterocytes, macrophages, and hepatocytes. Hepcidin binding induces ubiquitination of specific lysine residues on ferroportin. Ubiquitination leads to internalization and subsequent degradation of ferroportin within lysosomes. FPN: ferroportin; U: ubiquitination.*

### Other localizations and local roles of hepcidin

While hepatocytes are the primary source of plasma hepcidin, it is now recognized that other organs/tissues, such as the heart (285), kidney (286), macrophages (287), brain (288) and adipocytes (289), also contribute to its production. The presence of hepcidin in these cells and tissues suggests their participation in local iron homeostasis and potentially in specialized iron-related functions within their respective systems.

***Heart.*** Cardiomyocytes do express hepcidin and this expression is influenced by hypoxia and inflammation (290). Locally produced hepcidin reduces ferroportin expression and iron export

from cardiomyocytes, while specific deletion of hepcidin in cardiomyocytes or the expression of a hepcidin-resistant ferroportin isoform can lead to lethal cardiac dysfunction (291).

***Kidney***. Hepcidin expression has been shown in the distal nephron of the kidney (292), and particularly in the cortical thick ascending limb, in connecting tubules, and in the collecting ducts (286). Interestingly, the distal nephron expresses the DMT1 and ferroportin transporters at the apical and the basolateral membranes, respectively (293, 294),suggesting that the distal nephron may be involved in the reabsorption of nonheme iron from the tubular fluid and its subsequent return to the circulation. By acting on the expression and activity of ferroportin, hepcidin could thus modulate the reabsorption, renal accumulation, and blood transfer of iron. It has also been suggested that Escherichia coli may target renal hepcidin to attenuate its overall antibacterial activity during the early phase of a urinary tract infection (292).

***Pancreas.*** Hepcidin is expressed and directly regulated by iron in human pancreas, specifically in islet β-cells (295). Mouse models have shown that excess iron negatively impacts β-cell function , leading to islet iron accumulation, decreased insulin secretion and increased apoptosis (296), while iron deficiency protects against loss of β-cell function and diabetes (297). DMT1 and ferroportin are also expressed in β-cells. In a β-cell-specific DMT1 knockout mouse model, glucose-stimulated insulin secretion is reduced (298). This supports a role for β-cell-produced hepcidin in pancreatic iron homeostasis as well as in β-cell function.

***Adipocytes***. In obese patients, adipose tissue synthesizes hepcidin, with a correlation between hepcidin mRNA expression and inflammatory markers such as IL-6 and CRP (299). The production of hepcidin by adipose tissue can exacerbate iron-deficiency anemia, commonly observed in obesity (300).

***Macrophages***. Hepcidin production can be induced in the reticuloendothelial system and alveolar macrophages in response to inflammation or infection (301). Such induction of hepcidin may serve as a defense mechanism by restricting bacterial access to iron and inhibiting bacterial growth. In the liver, hepcidin can be induced by both bacterial and viral infections. In the brain and kidney, hepcidin induction can occur specifically in response to gram-negative bacteria, often mediated by the lipopolysaccharide (LPS)-toll-like receptor (TLR) pathway (292, 302). Such induction of hepcidin highlights its complex and multifaceted role in the immune response and host defense mechanisms.

Stomach parietal cells may also secrete hepcidin, playing a protective role against helicobacter pylori by increasing local acidity (303). Other studies have reported the production of hepcidin in the lungs (304), prostate (305), placenta (306), and retina (307), among others, possibly playing a role in defense against bacterial invasion.

### *1.2.2.3 Hepcidin regulatory pathway*

Hepcidin, the major regulator of iron homeostasis, is itself finely regulated. Multiple signals play a role in regulating hepcidin levels, including iron, inflammation, and erythropoiesis (308). These pathways are summarized in **(Figure 14).**

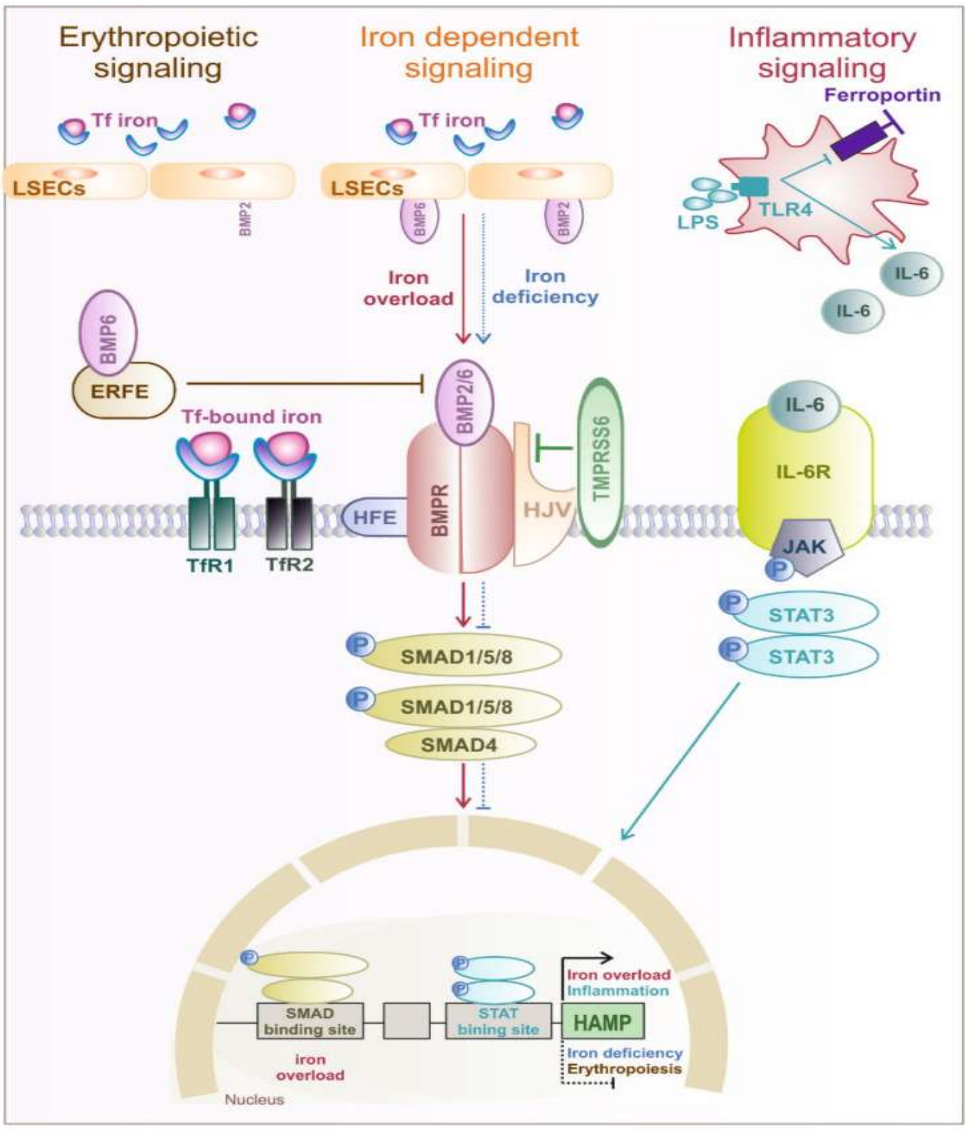


***Figure 14*: Major mechanisms regulating hepcidin expression.** *Hepcidin is regulated by various systemic stimuli, the three main ones being: (1) erythropoietic activity via Erythroferrone released by erythroblasts in response to erythropoietin. Erythroferrone inhibits the production of hepcidin by sequestering BMP 2 and 6, inhibiting activation of the BMP receptor and the BMP-SMAD pathway. (2) The body's iron status: increased intracellular (through increased BMP 2 and 6 and activation of the BMP receptor) and extracellular (through transferrin binding to its receptor) iron activates the BMP-SMAD pathway and therefore HAMP transcription. In case of intra or extracellular iron deficiency, the opposite occurs. (3) Inflammation, via IL-6 secreted by macrophages in response to the binding of bacterial lipopolysaccharides to their toll-like receptor 4, activating the JAK2-STAT4 pathway via the IL-6 receptor and inducing HAMP transcription. Activation of hepcidin by IL-6 also involves the BMP-SMAD pathway. At the protein level, matriptase-2 is a major negative regulator of hepcidin expression. One of the mechanisms is the degradation of hemojuvelin by matriptase-2, preventing the activation of the BMP receptor by its BMP ligands, which decreases hepcidin production. LSECs: liver sinusoidal endothelial cells; BMP: bone morphogenic protein; ERFE: erythroferrone; Tf: transferrin; TfR: transferrin receptor; HJV: hemojuvelin; TMPRSS6: matriptase-2; LPS: lipopolysaccharide; TLR: toll-like receptor; IL-6: interleukin 6; SMAD: Son of Mothers Against Decapentaplegic; STAT: signal transducer and activator of transcription; HAMP: hepcidin gene. Figure from Scaramellini et al (309).*

**Iron regulation**

As mentioned above, during iron deficiency, hepcidin expression declines, stabilizing ferroportin and facilitating iron absorption by duodenal enterocytes and iron release by macrophages. As a result, plasma iron availability increases. Conversely, in IO, hepcidin expression increases, causing ferroportin degradation and preventing iron export from enterocytes, macrophages, and hepatocytes. As a result, plasma iron levels drop.

The **Bone Morphogenetic Protein (BMP) - Son of Mother Against Decapentaplegic (SMAD) pathway** is the major pathway regulating hepcidin expression in response to iron (310). BMPs are a group of proteins belonging to the TGF-β superfamily involved in various biological processes, including embryonic development and bone growth. BMP ligands are initially produced as longer precursor proteins and undergo proteolytic cleavage to become mature and functional. There are more than twenty known BMP ligands, and several of them are produced in the liver, including Bmp2, Bmp4, Bmp5, Bmp6, and Bmp9 (311). Among these BMP ligands, **Bmp2 and Bmp6** have been identified as the key regulators of hepcidin expression (312, 313). In the liver, these two ligands are primarily produced by liver sinusoidal endothelial cells (LSEC) (311). This suggests a paracrine regulation of hepcidin expression, whereby BMP ligands produced by LSECs act on neighboring hepatocytes to modulate hepcidin production. BMP ligands interact with serine-threonine kinase receptors known as Activin-Like Receptors (ALK). There are two main types of ALK receptors involved in the BMP signaling pathway: BMP receptor type I (BMPRI) and BMP receptor type II (BMPRII) (314, 315). BMPRI is constitutively active, while BMPRII requires phosphorylation for activation (315). **Hemojuvelin (HJV**) is a co-receptor for BMPRs (316). In vivo, the BMP ligands BMP2 and BMP6 in dimeric form bind to the BMPR complex. The BMPR complex contains a BMPRI dimer, a BMPRII dimer, and co-receptors such as HJV and neogenin. Upon binding of BMP ligands to the BMPR complex, BMPRII hosphorylates BMPRI, which in turn phosphorylates the SMAD1/5/8 complex. The phosphorylated SMAD1/5/8 complex binds to the SMAD4 protein. This activated complex translocates into the nucleus and binds to BMP response elements (BMP-REs), inducing transcription of target genes such as HAMP (317) **(Figure 15).**

Under normal iron conditions, BMP2 is the main ligand responsible for regulating hepcidin expression (318), while BMP6 prevails under conditions of IO since its expression is induced by hepatic iron (319) through the action of transcription factor nuclear erythroid 2-Related Factor (essential for the modulation of gene expression by oxidative stress) (320). Furthermore, BMP2 expression can be regulated by reactive oxygen species (ROS) to increase hepcidin and limit iron toxicity (321). The importance of BMP6 in humans has been demonstrated through the identification of heterozygous nonsense mutations in patients. These mutations result in a decrease in the BMP-SMAD pathway and hepcidin synthesis, leading to moderate IO in affected individuals (322).

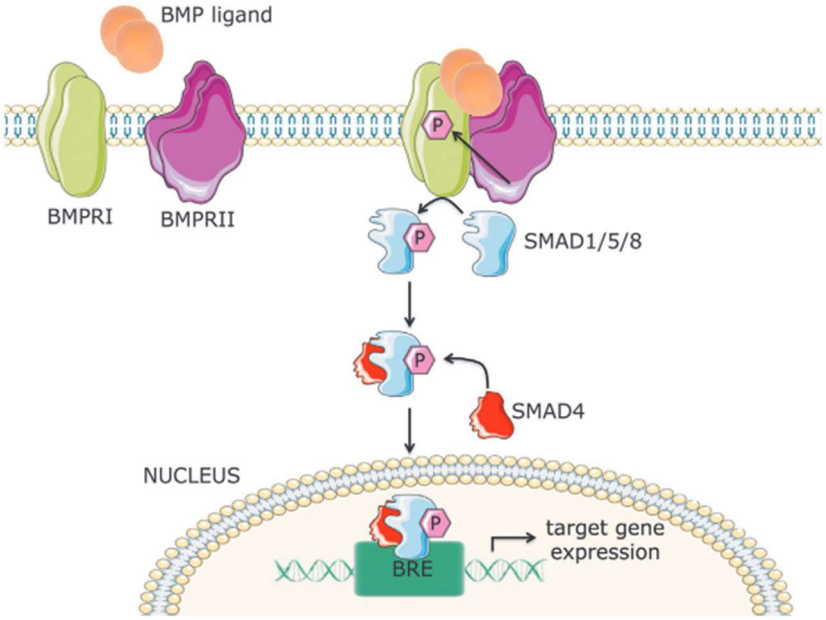


***Figure 15*: Mechanism of activation of the BMP-SMAD pathway by BMP ligands.** *BMP ligands, such as BMP2 and BMP6, bind to their respective BMP type II receptors (BMPRII) on the cell surface. This leads to the phosphorylation and activation of BMP type I receptors (BMPRI). Activated BMPRIs phosphorylate regulatory SMAD proteins, SMAD1, SMAD5 and SMAD8 (called SMAD1/5/8), which are the effector proteins of the BMP-SMAD pathway. The phosphorylated SMAD1/5/8 proteins form complexes with SMAD4. The interaction between phosphorylated SMAD1/5/8 and SMAD4 is essential for signaling downstream of the BMP-SMAD pathway. SMAD complexes, consisting of phosphorylated SMAD1/5/8 and SMAD4, translocate into the nucleus facilitated by nuclear import factors. Once inside the nucleus, SMAD complexes bind to specific DNA sequences called BMP reactive elements (BRE) into the promoter regions of target genes such as the hepcidin HAMP gene. BMP: bone morphogenetic protein; BMPR I and II: BMP type I or II receptor; BRE: bone morphogenetic protein reactive elements. Figure from Silvestry et al, 2019 (311).*

HVJ is present in the liver and skeletal muscle. In humans, mutations in the HVJ gene can lead to juvenile hemochromatosis, characterized by severe hepcidin deficiency and IO (323). This highlights the importance of HJV as a co-receptor of BMPRs in the regulation of hepcidin by iron. In addition to the membrane form, a soluble form of HVJ exists. It is the cleavage product of membrane HJV and is released into the extracellular environment (324). Soluble HJV acts as a negative regulator of hepcidin expression by competing with membrane HJV for binding to BMP ligands, preventing their interaction with the BMPR complex. Therefore, the BMP-SMAD pathway is not activated and hepcidin transcription is repressed. Membrane HJV and soluble HJV therefore have opposing roles, the first inducing the expression of hepcidin and the second repressing it.

Hepcidin expression is also inhibited by SMAD7 and matriptase-2, through their effects on the BMP-SMAD pathway. SMAD7 was identified using a siRNA screen in HuH7 cells, a human hepatocarcinoma cell line. SMAD7 acts by binding to BMPRI and competing with SMAD1/5/8 for phosphorylation, thereby inhibiting the BMP-SMAD pathway (325).

**Matriptase-2**, a serine protease encoded by the TMPRSS6 gene, is primarily expressed in the liver. The major role of matriptase-2 as negative regulator of hepcidin production was discovered in

2008 when Du *et al.* induced chemical mutations in its gene (mask mice), resulting in the loss of its proteolytic domain (326). In mask mice lacking matriptase-2, microcytic hypochromic anemia, low SeFe and reduced transferrin saturation (Tsat) are observed. In vitro studies have demonstrated that matriptase-2 plays a role in regulating hepcidin expression by cleaving HJV. By cleaving HJV, matriptase-2 attenuates the activation of the BMP-SMAD pathway, resulting in reduced hepcidin expression (327). However, it is surprising that mask mice and TMPRSS6-deficient mice showed reduced rather than increased total hepatic HJV protein content (328). Recently, matriptase-2 has been proposed to also regulate hepcidin expression through the cleavage of several components of the BMP-SMAD signaling pathway, such as BMPRIs, HFE and TfR2, independently of HJV cleavage (329, 330). This might provide an explanation for the lack of elevated HJV levels in mask mice. In humans, mutations in the TMPRSS6 gene lead to a hereditary autosomal recessive disease known as iron-refractory iron-deficiency anemia (IRIDA) (331). These matriptase-2 mutations induce an abnormally high level of ferroportin-degrading hepcidin, which blocks iron uptake by enterocytes and iron recycling by macrophages (326). The amounts of iron are then insufficient to meet the erythropoietic demand and consequently lead to congenital microcytic and hypochromic anemia. SF is normal or high (reflecting iron trapped in macrophages) and Tsat is collapsed (<5%; corresponding to the absence of SeFe available for erythropoiesis) (332). Most IRIDA patients do not respond to oral iron treatment, requiring IV iron to correct their anemia by circumventing the intestinal barrier (333). Moreover, Meynard *et al.* demonstrated that BMP6 and iron not only induce hepcidin but also matriptase-2 expression (334). This regulation could serve as a negative feedback inhibition to avoid excessive hepcidin induction by iron. Finally, maptriptase-2 expression increases during hypoxia, decreasing membrane HJV as well as the response of the BMP-RE proximal to the hepcidin promoter. Maptriptase-2 regulation by hypoxia depends on HIF-1α and HIF-2α, through a HRE in the promoter of the TMPRSS6 gene (335). However, the inhibition of hepcidin expression by hypoxia does not depend solely on maptriptase-2, because TMPRSS6 -/- mask mice inhibit hepcidin expression in hypoxic conditions (336).

**The HFE/TfR1/TfR2 pathway** is another important pathway involved in the regulation of hepcidin expression by Tsat. The HFE gene encodes a transmembrane glycoprotein that is classified as a non-classical major histocompatibility complex (MHC) class I protein. Mutations in the HFE gene are the common mutations associated with hereditary hemochromatosis (337). TfR1 is responsible for the uptake of iron-bound transferrin into cells, while TfR2 is involved in signaling iron levels to regulate hepcidin expression (338). When iron-bound transferrin (holo-Tf) is absent or low, HFE and TfR1 form a complex (339). However, as transferrin becomes saturated with iron, TfR1 exhibits a higher affinity for holo-Tf than for HFE, leading to the dissociation of the HFE-TfR1 complex. The released HFE then binds to TfR2. The formation of the

HFE-TfR2 complex, independently of Bmp6 induction, activates the phosphorylation of SMAD1/5/8 proteins (340). These phosphorylated SMADs can translocate into the nucleus and enhance hepcidin transcription **(Figure 16).**

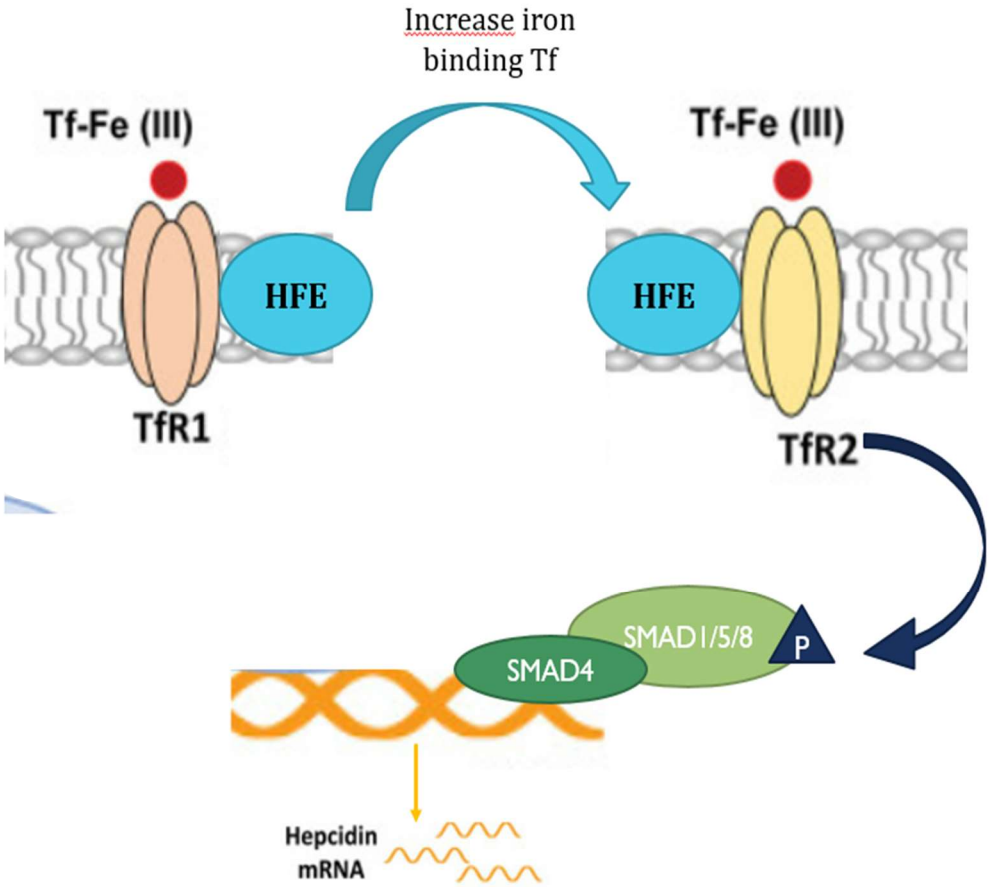


***Figure 16*: The HFE/TfR1/TfR2 pathway.** *As transferrin saturation increases, the affinity of HFE binding to TfR1 decreases, while it increases for TfR2. Binding of HFE to TfR2 leads to phosphorylation of the SMAD1/5/8 protein complex, which binds to SMAD4. The SMADs complex migrates into the nucleus to activate expression of the HAMP gene encoding hepcidin. BMP: bone morphogenetic protein, TfR1: transferrin receptor 1, TfR2: transferrin receptor 2; P: phosphorylation. Figure adapted from Zheng et al 2023 (341).*

**Inflammatory regulation**

Infection and inflammation induce the production of hepcidin (342), which inhibits iron efflux from enterocytes and macrophages, thereby decreasing SeFe concentrations (343). It has been proposed that this rapid defense mechanism could provide an advantage during certain bacterial (344) and fungal infections (345) by limiting the availability of iron for pathogens. Indeed, some pathogens rely on iron for their growth and survival and have developed sophisticated mechanisms to acquire iron from the host (345-347). A protective role of hepcidin and hypoferremia is established for siderophilic bacteria, including Vibrio vulnificus (344) and Yersinia enterocolitica, which can be lethal in patients with hepcidin deficiency and subsequent hemochromatosis (348). Bacteria and fungi maintain iron homeostasis by synthesizing iron-

chelating molecules called siderophores that enhance their virulence (349). IL-6 production in mice infected with Aspergillus fumigatus could modulate siderophore production while neutrophils synthesize lipocalin-1, which sequesters fungal siderophores (345). Hence, topical application of lipocalin-1 restricts fungal growth in vivo while exogenous iron promotes fungal growth in infected mice (345). Conversely, despite hypoferremia, intracellular organisms residing within macrophages, such as Salmonella Typhi, may exhibit increased pathogenicity upon hepcidin activation, which sequestrates iron within macrophages (350).

TLRs are key sensors of the innate immune system. They accept pathogen-associated molecular patterns (PAMPs) and control the hypoferremic response of the host. For example, LPS is a cell wall component of gram-negative bacteria recognized by TLR4. Hepcidin has similarities to antimicrobial peptides induced by LPS. Injection of LPS in mice (134, 272, 351) and in healthy subjects (352) induces the transcription of hepcidin in the liver and decreased SeFe levels. LPS induces the production of various pro-inflammatory cytokines, such as IL-6, IL-1β and TNF-α (311). IL-6 is one of the main cytokines involved in the induction of hepcidin during inflammation (134). Indeed, in healthy subjects, IL-6 injection increases the amount of urinary hepcidin (134). During inflammation, IL-6 binds to its receptor, the IL-6 receptor, which phosphorylates JAK2 (p-JAK2). p-JAK2 in turn phosphorylates the transcription factor STAT3, which translocates into the nucleus and binds to the STAT3-Responsive Element (STAT3-RE) binding site present in the hepcidin promoter, thereby to induce its transcription (353, 354). Moreover, the BMP-SMAD pathway is also required to produce hepcidin through the IL-6 pathway. Indeed, hepcidin is not induced in response to IL-6/LPS in mice deleted for either SMAD4 (355) or ALK3 (356). The importance of the proximal BMP-RE in addition to the STAT-RE in the hepcidin promoter during inflammation has also been shown in vitro (357, 358). In addition, inflammation via activin b, a cytokine of the TGF-β family that can bind to the BMP receptor, further activates hepcidin transcription (359). This shows a close link between the BMP-SMAD pathway and the IL-6/STAT-3 pathway during inflammation.

Hepcidin can also be induced in vivo and in vitro via other TLRs than TLR4. Indeed, the ligands of TLR3, TLR7/8 and TLR9 induce the expression of hepcidin, while Pam3CSK4 and ImQ, the respective ligands of TLR 1/2 and TLR3, decrease ferroportin expression (360, 361).

Moreover, Meynard *et al.* showed that inflammation via IL-6 not only induces hepcidin expression, but also inhibits matriptase-2 expression by decreasing STAT-5 phosphorylation (362). Analysis of the TMPRSS6 promoter shows the presence of a STAT5-RE, suggesting that STAT5 directly regulates the expression of matriptase-2. Given that TMPRSS6 functions as an inhibitor within the BMP-SMAD pathway and hepcidin expression, this mechanism entails a negative feedback loop involving TMPRSS6 modulated by inflammation through the BMP-SMAD pathway. Consequently, this intricate interplay contributes to the induction of hepcidin production in response to IL-6.

Besides hepcidin-induced hypoferremia, the anemia of inflammation arises from multiple other factors, including plasma expansion, diminished RBC survival, reduced EPO production and interferon-mediated inhibition of erythroid precursors (129).

**Erythropoietic regulation: Erythroferrone**

Erythropoiesis inhibits hepcidin expression more efficiently than anemia or hypoxia (363). Studies in mice (364) and humans (365, 366) have provided evidence for the link between hepcidin suppression and erythropoiesis. In experiments with healthy volunteers, subcutaneous injections of EPO or phlebotomies induced a rapid, persistent drop in hepcidin levels in plasma (367, 368). Other studies in mice have demonstrated that irradiation, which destroys hematopoietic cells, prevents the inhibition of hepcidin expression by EPO (369). These reports suggest that a factor associated with erythropoiesis and produced by hematopoietic cells, possibly circulating in the bloodstream, is responsible for hepcidin suppression during erythroid stress.

Several proteins have been proposed as regulators of hepcidin expression by erythroid stress. Growth Differentiation Factor 15 (GDF-15), a member of the TGF-β family, is produced during erythroblast maturation and has been associated positively with EPO. Plasma levels of GDF-15 are elevated in non-transfusion-dependent β-thalassemia and in congenital dyserythropoietic anemia and have been suggested to suppress hepcidin expression, contributing to the IO observed in these pathologies (370-372). However, GDF-15 knockout mice still suppress hepcidin expression after phlebotomy (373), while only exceptionally elevated GDF-15 concentrations reduce hepcidin levels ex vivo (374). Hence, GDF-15 does not appear to be a physiological regulator of hepcidin during normal erythropoiesis, but could play a role in stress erythropoiesis, for instance resulting from oxidative stress in β-thalassemia (375). It is likely that GDF-15 is primarily a marker of ineffective erythropoiesis in disorders such as β-thalassemia, congenital dyserythropoietic anemias, or, to a lesser extent, pyruvate kinase deficiency (376, 377). Twisted gastrulation BMP signaling modulator 1 (TWGS1), another hormone secreted by erythroblasts, has been also incriminated, but EPO administration or phlebotomies do not induce its transcription (378).

In 2008, Pinto demonstrated the regulation of hepcidin expression by EPO, in a dose-dependent manner, suggesting the contribution of a transcription factor in the response of hepcidin to EPO (379). Later, Kautz *et al.* identified the erythroid regulator as erythroferrone or ERFE (380). Using ERFE knockout mice, they demonstrated suppression of hepcidin expression during the initial 24 hours after phlebotomy or in response to EPO administration (380). In response to EPO, ERFE is produced by erythroid precursors in the BM and spleen, and its production is mediated by the signaling molecule STAT5 in mice (380) and humans (381). Recent research has revealed that ERFE acts by sequestering BMP ligands, specifically BMP2 and BMP6, preventing them from forming a heterodimer complex (382) and interacting with ALK3, one of the BMP type I receptors,

thereby blocking the BMP-SMAD pathway and subsequent hepcidin expression (382). By inhibiting the production of hepcidin, ERFE facilitates iron absorption and mobilization, making it available for active erythropoiesis **(Figure 17).**

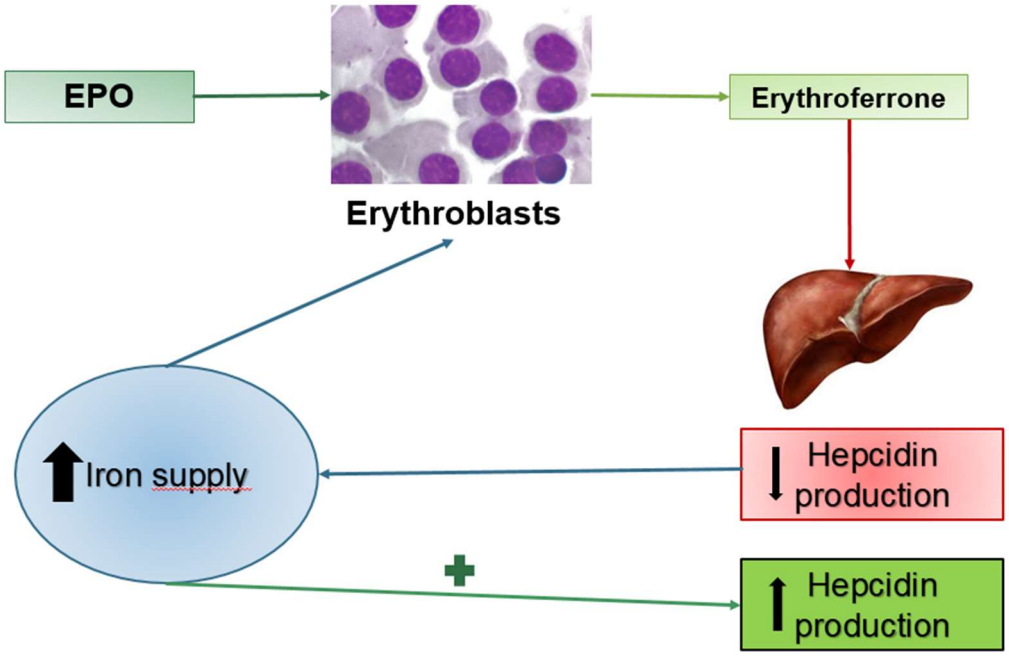


***Figure 17*: Erythroid regulation of hepcidin mediated by erythroferrone.** *In conditions of increased erythropoiesis consecutive to anemia or hypoxia, hepcidin synthesis is greatly reduced. Erythroferrone, released by proliferating erythroblasts in response to erythropoietin, will inhibit the production of hepcidin to increase iron supply available for active erythropoiesis. Once iron availability is increased in the blood, hepcidin synthesis via the BMP-6 pathway increases again and closes this regulatory loop.*

In addition, matriptase-2 plays a crucial role in the regulation of hepcidin through erythropoiesis. Indeed, Nai *et al.* have shown that EPO administration increases ERFE and TMPRSS6, while inhibiting hepcidin expression in wild-type mice, but not in TMPRSS6 -/- mice (mask mice) (383). Therefore, the inhibition of hepcidin by ERFE depends on the presence of matriptase-2, which acts to attenuate the BMP-SMAD signaling pathway and, subsequently, hepcidin expression. Although, TMPRSS6 -/- mask mice are resistant to EPO treatment, EPO injections induce extramedullary erythropoiesis and increase spleen size, but the systemic iron deficiency (due to excess hepcidin) hampers erythroblast maturation (224). After systemic injection of iron dextran, RBC parameters normalize, and the response to EPO is restored, but elevated hepcidin expression persists (221). The lack of EPO effect on hepcidin expression in iron overloaded mask mice suggests that downregulation of hepcidin synthesis by erythropoietic activity occurs only when iron levels are within the physiological range (225). It is acknowledged that hyper-activation of the BMP-SMAD pathway, linked to the absence of matriptase-2, outweighs the inhibition of hepcidin by erythroid stress (the impact of ERFE) in mask mice. Overall, these findings suggest that two signaling

pathways are simultaneously activated in response to EPO; the ERFE pathway (via BMP6) and the matriptase-2 pathway. However, the involvement of matriptase-2 has not been widely accepted. Indeed, the study of Aschemeyer *et al.* indicated that TMPRSS6 -/- mice exhibit high levels of hepcidin, but the additional absence of ERFE has only a minor effect on their phenotype (384).

Animal models of inflammation and thalassemia have demonstrated that this negative erythropoietic regulator of hepcidin dominates the positive effect of inflammation (385) or IO (386). We also previously showed that, after autologous hematopoietic cell transplantation (HCT), the major determinants of hepcidin production were iron stores and erythropoietic activity while inflammation exerted a minor role (387).

**Other regulators**

Liu *et al.* have recently shown that ***lactate*** binds to soluble adenylyl cyclase in normal mouse hepatocytes, influencing systemic iron homeostasis (388). They demonstrated in vitro and in vivo that this interaction led to elevated levels of cyclic adenosine monophosphate, thus activating the PKA-SMAD1/5/8 signaling pathway and promoting hepcidin transcription. This induction of hepcidin was suggested to reduce the concentration and activity of ferroportin, elevating intracellular iron and facilitating mitochondrial clearance of lactate. This regulation has been confirmed in wild-type mice and those with disruptions in iron homeostasis. Furthermore, lactate regulates hepcidin in individuals at rest and subjected to intense exercise, thereby generating elevated lactate levels. This establishes a connection between hyperlactatemia and iron deficiency, explaining the anemias observed in athletes (389) and patients with lactic acidosis as during cancer (390), sepsis (391) or diabetes (392).

Sardo *et al.* have identified ***FGL1***, a hepatokine, as a novel suppressor of hepcidin, induced in the liver during the recovery phase from anemia and in thalassemic mice in response to hypoxia (393). FGL1 effectively suppressed hepcidin both in vitro and in vivo. When FGL1 was deleted in mice, baseline and post-phlebotomy hepcidin levels were higher. FGL1 functions by directly interacting with BMP6, thereby impeding the canonical BMP-SMAD signaling pathway responsible for regulating hepcidin transcription.

***Other less important regulators*** have been reported such as positive regulation by ER stress (394) or negative regulation by testosterone on hepcidin expression (395), explaining differences in hepcidin expression levels between male and female mice. Testosterone would act through the EGF (Epidermal Growth Factor) receptor that inhibits the BMP-SMAD pathway (134).

### 1.2.3 Iron disorders

Hepcidin regulation is thus central to systemic iron metabolism. Any dysregulation of its production will modify iron availability, both within cells and in the blood. A lack of iron can be responsible for disordered erythropoiesis and anemia, while IO can be harmful due to its toxicity. SF represents body iron stores and is commonly measured in clinical practice (241). Indeed, a study performing phlebotomies in healthy subjects showed a correlation between SF and iron stores, with 1 ng/mL ferritin corresponding approximately to 8 mg/kg iron stores (242). Under physiological conditions, SF is mostly composed of the L-chain, is low in iron and partly glycosylated, indicating mainly hepatocyte secretion (235, 236). Normal SF levels are between 20-200 ng/mL in women and 20-300 ng/mL in men. A value <20 ng/mL is 100% specific for absolute iron deficiency (no iron stores), whereas high values indicate IO. However, hepatic cytolysis, inflammation, renal insufficiency, hyperthyroidism, and certain tumors… are associated with an inappropriate elevation of SF, which does not reflect real IO but only ferritin release or secretion in the blood (396). Interpretation of elevated SF levels can therefore be difficult.

Another blood parameter, i.e., Tf saturation (which can be calculated as the ratio serum iron/transferrin*1.42) is used in current practice to provide information on immediate iron availability for erythropoietic and other cells. A normal Tsat is between 20-40%. A Tsat < 20% demonstrates insufficient iron supply to meet the demand (as found in absolute as well as functional iron deficiency). Conversely, a high Tsat > 45% defines iron availability exceeding iron demand and is found for instance in genetic hemochromatosis or erythroid marrow aplasia. Iron and RBC parameters encountered in normal conditions or in the case of iron deficiency or hyperferritinemia (with or without IO) are listed in **Table 1**.

***Table 1:** Iron and red blood cell parameters in physiological and pathological conditions.*

| Iron parameters | Normal range | Absolute iron deficiency | Fonctional iron deficiency | Hyperferritinemia without iron overload | Iron overload: genetic hemochromatosis | Iron overload: transfusions and ineffective erythropoiesis |
|---|---|---|---|---|---|---|
| Serum iron (µmol/L) | 8-30 | ↓ | ↓ | ↑ | ↑ | ↑ |
| Transferrin saturation (%) | 20-45 | < 20 | < 20 | 20-45 | > 45 | > 45 |
| Serum ferritin (ng/mL) | 20-200 (F) or 20-300 (M) | < 20 | > 200 (F) or > 300 (M) | > 200 (F) or > 300 (M) | > 200 (F) or > 300 (M) | > 200 (F) or > 300 (M) |
| Red blood cells ($10^6$/µL) | 4.0-5.2 (F) or 4.4-5.7 (M) | ↓ | ↓ | normal | normal | ↓ |
| Hemoglobin (gr/dL) | 12.0-15.0 (F) or 13.5-17.0 (M) | ↓ | ↓ | normal | normal | ↓ |
| Hematocrit (%) | 36-45 (F) or 40-52 (M) | ↓ | ↓ | normal | normal | ↓ |
| Mean corpuscular volume ($\mu^3$) | 80-100 | < 80 | < 80 | normal | normal | variable |
| Hemoglobin per red blood cell (pg) | 27-33 | < 27 | < 27 | normal | normal | normal |
| Hypochromic red blood cells (%) | < 5 | >> 5 | >> 5 | normal | normal | normal |
| Reticulocytes ($10^3$/µL) | 25-100 | < 25 | < 25 | normal | normal | variable |
| Hemoglobin per reticulocyte (pg) | 26-31 | < 26 | < 26 | normal | normal | normal |

### *1.2.3.1 Iron deficiency*

A distinction is made between absolute iron deficiency (i.e. no iron stores in macrophages) and functional iron deficiency (normal or increased iron stores but insufficient storage iron release to meet the demand of erythroblasts; the BM ultimately lacks iron).

**Absolute iron deficiency (AID)**

Nutritional iron deficiency is the most prevalent cause of anemia (>50% of global cases), affecting approximately 1 billion people worldwide in 2019 (397). It occurs when iron intake is not sufficient to meet physiological needs or when tissue iron stores are depleted due to bleeding or poor iron absorption at the duodenal level. In the absence of inflammation (which results in increased ferritin through increased hepcidin via the IL-6 pathway), absolute iron deficiency anemia (IDA) is characterized by low SF concentration (<20 ng/mL), indicating depleted body iron stores, low Tsat (<20%), and increased soluble TfR1 (this soluble form correlates with the total mass of TfR on iron-deficient erythroblasts) (398). AID is hypochromic and microcytic (defined by an Hb<12 g/dL in women and <13 g/dL in men with a mean corpuscular volume (MCV) <80 fl). Hepcidin in the serum of patients with iron deficiency anemia is almost always undetectable (399). Iron deficiency manifests with a broad clinical picture that may or may not be associated with anemia depending on the degree of deficiency and time since deficiency (400). Iron deficiency anemia preferentially affects children and pregnant women, with a higher prevalence in developing countries. Oral iron supplementation is the first-line treatment.

**Functional iron deficiency (FID)**

Functional iron deficiency is second in terms of frequency, after AID. FID occurs in patients with inflammation because increased hepcidin expression in response to the inflammatory reaction induces iron sequestration in macrophages. The iron needs for erythropoiesis are no longer satisfied, which leads to the progressive development of ACD. FID is normocytic at first, then microcytic in the long run. SeFe is low, SF remains normal or increases, indicating that iron stores are preserved. Iron retained in macrophages inactivates IRPs, leading to increased transcription of ferritin, also increasing SF. This mechanism is found in inflammatory pathological situations such as infection, cancer, chronic renal failure, or autoimmune diseases (401). Treatment of this anemia by oral iron intake is not effective; indeed, the overexpression of hepcidin blocks iron absorption by enterocytes and iron recycling by macrophages. Consideration should therefore be given to treating the inflammation; when such treatment is effective, there is usually a return to normal blood parameters.

Absolute iron deficiency may occur in patients with ACD. A ferritin value < 100 µg/L among patients with chronic inflammation indicates an absolute deficiency since ferritin levels are

"falsely" elevated by chronic inflammation. These patients can therefore benefit from an IV iron supply to support erythropoiesis, while oral iron is generally poorly absorbed. As discussed under *1.1.3.5*, EPO therapy is effective in cancer-related inflammatory anemias. However, it is essential in clinical practice to first treat any absolute iron deficiency before starting ESAs. In fact, re-establishing a correct supply of IV iron has been shown to improve EPO efficacy, reduce its use and lower its economic cost (402).

### *1.2.3.2 Iron overload*

IO translates into elevated SF levels mostly associated with increased Tsat. Hyperferritinemia is defined by a blood ferritin level above 200 ng/mL in women (non-menopausal) and 300 ng/mL in men (403). The main etiologies of hyperferritinemia (with or without IO) are summarized in **Table 2**.

***Table 2*: Main causes of hyperferritinemia.** *NASH: nonalcoholic steatohepatitis; RBC: red blood cells. Table adapted from Sandnes et al (396).*

| Categories | Etiology |
|---|---|
| **Hyperferritinemia without iron overload** | **Common causes** |
| | Inflammation<br>Cancer<br>Hepatocyte damage<br>Diabetes and insulin resistance |
| | **Rare causes** |
| | Gaucher disease |
| **Hyperferritinemia with or without iron overload** | Metabolic syndrome - NASH- Chronic liver disease<br>Alcohol consumption |
| **Hyperferritinemia with iron overload** | **Primary causes** |
| | ***Without anemia*** |
| | Genetic hemochromatosis (HFE and non-HFE)<br>Ferroportin disease type II |
| | ***With anemia*** |
| | Ferroportin disease type I<br>Aceruloplasminemia<br>Atransferrinemia<br>DMT1 mutation |
| | **Secondary causes** |
| | ***Without anemia*** |
| | Excessive intravenous iron |
| | ***With anemia*** |
| | Chronic RBC transfusions<br>Ineffective erythropoiesis<br>*Thalassemia and other hemoglobinopathies*<br>*Chronic hemolytic anemia*<br>*Myelodysplastic syndrome* |

Increased ferritin with a normal Tsat is associated with true IO in < 10% of cases (404). **Hyperferritinemia without IO** therefore represents most situations (396). The causes can be secondary to ferritin release in the blood (as encountered in hepatocyte lysis or in inflammation), or be primary, of genetic origin (mutation of the ferritin gene).

**IO** occurs when total body iron exceeds ~3–4 g. In practice, as soon as the transferrin saturation exceeds 45%, there is a formation of non-transferrin bound iron (NTBI). Such free SeFe rapidly penetrates inside cells, where it may lead to an increased labile iron pool (LIP). Such free iron is oxidized by the Fenton reaction and leads to the production of ROS which cause cellular damage, including the destruction of the endoplasmic reticulum, mitochondria as well as DNA damage(405). This leads to cell apoptosis and progressively to organ failure (406).

Iron can theoretically accumulate in all cells and organs, but the first and most frequently affected are liver hepatocytes. Such IO in the liver can ultimately lead to cirrhosis and hepatocellular insufficiency as well as hepatocarcinoma (405). The other affected organs are the heart, with a risk of heart failure and arrhythmia; the pancreas, with the onset of diabetes; and the gonads, pituitary, thyroid, and parathyroid glands, leading to multiple endocrine insufficiencies. Advanced organ damages are preceded by signs of incipient IO, such as asthenia, painful arthralgias (typically joint pain in the 2nd and 3rd phalanges through the development of microcrystalline arthropathy) and hepatic cytolysis (407).

NTBI is not measured in routine clinical practice. It is the combination of Tsat and ferritin assays that points to IO. A Tsat >45% indicates iron supply exceeding iron demand and therefore suggests the formation of free toxic iron. Ferritin assays are routinely used for quantifying excessive iron stores but can be falsely elevated in many conditions such as inflammatory disorders, hepatic cytolysis, or the metabolic syndrome (396). In cases where ferritin values seem unreliable, magnetic resonance imaging (MRI) is the gold standard for quantifying true liver IO (408, 409).

**IO can be primary (genetic) or secondary**. Commonly, **hereditary genetic hemochromatosis** is due to insufficient production of hepcidin or to resistance to the hepcidin signal secondary to a mutation of one of the proteins involved in the regulation of hepcidin by the iron pathway (410). The diagnosis is based on iron parameters (Tsat > 45% and ferritin increase > 200 ng/mL in women or 300 ng/mL in men) and must be confirmed by genetic testing. The most common form of hemochromatosis in Caucasians is due to homozygous C282Y mutation in the HFE gene (411). This mutation has a prevalence of 1/200 in the northern European population (412), leading to a defect in HFE, the essential co-receptor of the TFR, and to the activation of the BMP-SMAD pathway by plasma iron bound to transferrin. Without HFE, there is no production of hepcidin, and macrophages and enterocytes no longer can retain iron, which is thus massively released into the circulation. The first biological abnormality that appears is an increased Tsat, and later around

the age of 20, SF increases because of the accumulation of iron in hepatocytes (406). There are also non-HFE forms of hemochromatosis that are rarer, multi-ethnic, and affect younger patients with more severe hepatic IO (323, 413). Non-HFE forms result from mutations in HVJ, TfR2, hepcidin or ferroportin. Patients with ferroportin (SLC40A1) mutations fall into two classes, with distinct phenotypes depending on the mutation they possess. The first type of autosomal dominant mutation produces a loss of function of ferroportin, resulting in ferroportin disease. FPN disease is characterized by a reduction in FPN expression on the plasma membrane, leading to reduced cellular iron export and iron accumulation in cells, particularly in spleen macrophages (414). In the liver, iron accumulation is primarily seen in Kupffer cells, in contrast to the hepatocyte-dominated periportal distribution observed in the early stages of HFE-related hemochromatosis. These patients therefore have a clinical (splenic>hepatic IO) and biological (normal or low plasma iron and Tsat) presentation that is different from classical hemochromatosis. Ferritin levels are very high with a normal or low Tsat (because iron is blocked in macrophages) and patients develop microcytic anemia due to functional iron deficiency, particularly when phlebotomies are performed. The second type of ferroportin mutations, characterized by an autosomal recessive mode of inheritance, is caused by a missense mutation in SLC401 that generates a gain-of -function FPN protein with reduced responsiveness to hepcidin (415). The phenotype is quite like that associated with HFE, TfR2, HJV and hepcidin mutations, with elevated transferrin saturation and periportal iron deposition in liver hepatocytes. The treatment here also consists in phlebotomies (416).

**IO may also be secondary to chronic transfusions**. In fact, each RBC transfusion contains approximately 200 milligrams of iron. Therefore, if a patient is transfused with 2 units per month, he or she accumulates 5 grams of iron within one year! IO secondary to chronic transfusions occurs after of the transfusion of 10 to 20 units of RBCs, with accumulation of free iron that can lead to cellular damage and organ failure (417).

Finally, another common cause of secondary (non-genetic) IO associated with anemia may be induced by a mechanism of **ineffective erythropoiesis** encountered in various hematological diseases. Indeed, it has been shown that in nontransfused and transfused β-thalassemia (418), hemolysis (immune and non-immune) (419) and MDS (420), the inhibition of hepcidin production leads to increased intestinal iron absorption and aggravates the pre-existing IO (421). In these diseases, erythroblasts proliferate sometimes considerably but die prematurely of apoptosis, secreting a lot of ERFE and releasing the iron contained in their hemoglobin (419, 422). In these conditions, the erythropoiesis-driven ERFE pathway inhibiting hepcidin production is more powerful than the iron-induced mechanism stimulating hepcidin production via the BMP-SMAD pathway. This leads to IO via overall reduced hepcidin production (423) **(Figure 18)**.

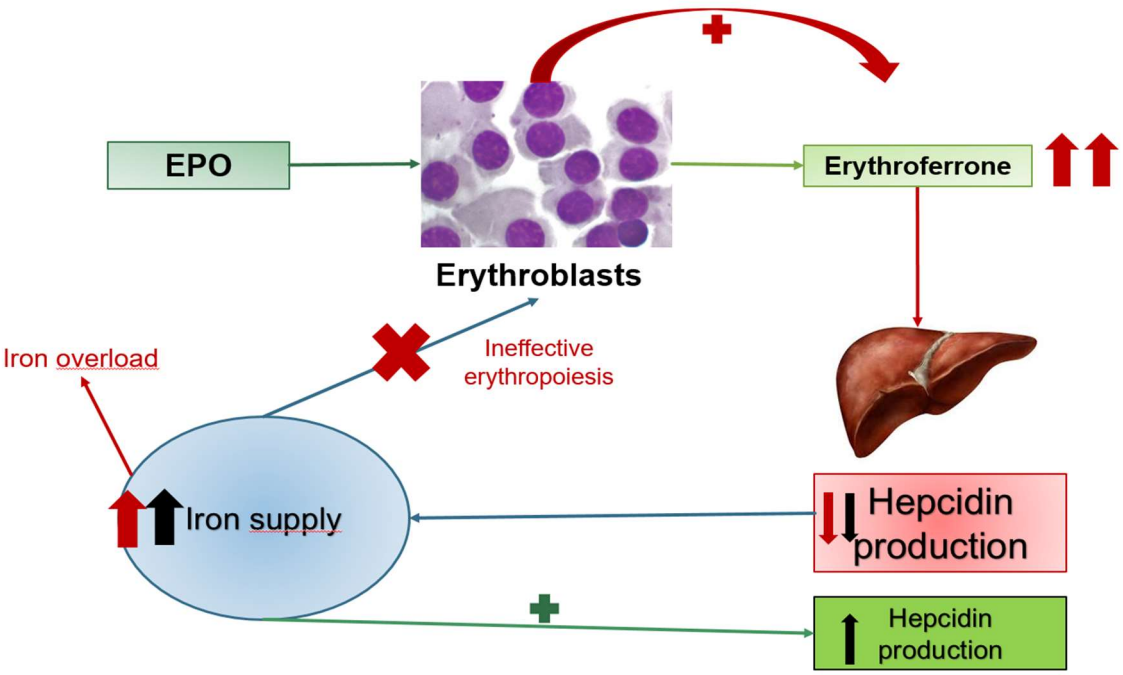


***Figure 18*: Ineffective erythropoiesis in β-thalassemia or myelodysplastic syndrome**. *Erythroblasts proliferate but die prematurely of apoptosis, leading to anemia. Active erythropoiesis secretes a lot of erythroferrone, which inhibits hepcidin production, leading to continuous entry of iron into the body. Since iron is not fully used for erythropoiesis, it accumulates in organs such as the liver, heart, pancreas and gonads. The positive feedback of iron on the production of hepcidin is less powerful than the inhibitory effect of erythroferrone. This results in overall iron overload. EPO: erythropoietin.*

On the other hand, studies have highlighted the role of GDF-15 in hepcidin inhibition among thalassemic patients, with negative associations between these two parameters (424). However, this relationship remains controversial (425). Indeed, the variability in GDF-15 levels and other parameters (such as Hb, hepcidin, and ferritin) could be explained by the different therapeutic regimens used in the management of thalassemic patients. For instance, lower GDF-15 levels were measured following appropriate chelation protocols and in patients undergoing hydroxyurea therapy (426), likely due to reduced ineffective erythropoiesis. On the other hand, under-transfused patients (pre-transfusion levels < 8 g/dl) displayed higher erythroid activity and ineffective erythropoiesis (427). As mentioned in section 1.2.2.3, GDF-15 appears to be primarily produced in situations of ineffective erythropoiesis.

IO in patients with β-thalassemia or MDS has been shown to be associated with poorer survival (428, 429). Subsequent studies demonstrated that the use of iron chelators in these patients could improve survival (430, 431). It is therefore important to reduce excess iron not by phlebotomies (since patients are anemic) but by using oral iron chelators such as Deferasirox, which is the oral form most used in clinical practice (432).

Sickle cell disease (SCD) results from specific homozygous mutations (HbSS) or compound heterozygous mutations involving hemoglobin S (HbS) combined with hemoglobin C (HbSC) or

beta-thalassemia (HbS/beta-thalassemia), leading to RBC deformation under certain conditions, causing vaso-occlusion, inflammation and hemolysis (433). The regulation of iron metabolism is not fully understood in SCD (434). In patients with SCD, there is both some degree of ineffective erythropoiesis (illustrated by slightly elevated GDF15 and increased EPO and sTfR levels) and significant inflammation explaining IL-6-driven hepcidin production (433, 435, 436). ERFE levels are elevated, although less so than in other diseases, but show no correlation with hepcidin (436, 437). RBC transfusions are restricted to life-threatening emergencies to reduce the risk of transfusion reactions (438), partly explaining less prominent IO compared to beta-thalassemia (439). In addition, suppression of hepcidin by ERFE may be partially countered by its induction by IL-6, which could also limit the development of iron overload. However, contradictory evidence exists regarding the correlations between hepcidin on the one hand, and both inflammation and erythropoiesis on the other, illustrating the complex interplay of factors that may occur differentially in individual patients (440, 441).

# 2. Allogeneic hematopoietic stem cell transplantation

## 2.1 Transplant procedures and outcomes

Allogeneic HSCT (allo-HSCT) is a complex therapeutic procedure involving the infusion of hematopoietic stem cells from a donor to a recipient who has undergone a short period of conditioning with chemotherapy and/or radiotherapy (442). Although early attempts were largely unsuccessful, the procedure has evolved considerably over recent decades (443). In 2019, the European Society for Bone and Marrow Transplantation reported that over 40,000 allo-HSCT had been performed at 700 international centers, representing an exponential growth rate, with over 800,000 HSCTs recorded in 30 years (444)! The World Blood and Marrow Transplant Network has also recorded increasing allo-HSCT activity since 1957 (445). Historically reserved for the treatment of acute leukemia, chronic myeloid leukemia and aplastic anemia, the indications for allo-HSCT have gradually been extended to other hematological malignancies (MDS, myeloproliferative neoplasia, lymphoid hemopathies…) (446), hemoglobinopathies (thalassemia β major, sickle cell disease) (447), severe congenital immune deficiencies and other severe diseases (448).

Recipient eligibility for HSCT is discussed according to age, comorbidities, and donor availability (449). The choice of a donor is based first and foremost on Human Leukocyte Antigen (HLA) histocompatibility between donor and recipient. The HLA system comprises over 200 genes located in a region of the short arm of chromosome 6 and divided into 3 parts (HLA class I, II and III) (450, 451). The genes of the HLA system are mainly involved in the immune response and have the common feature of being polymorphic and co-dominantly expressed. Transmission to offsprings respects complete haplotypes but crossovers are sometimes encountered. HLA-compatible donors may be related (familial) to the recipient. They can be "Genoidentical" (siblings with a 10/10 HLA match for HLA-A, B, C, DRB1 and DQB1) or "Haploidentical" (parents, children, siblings or other family members sharing a haplotype, with a resulting match of at least 5/10 HLA antigens) (452). The probability of having a 10/10 HLA-matched sibling is only 30%. In that case, a haploidentical donor can be selected or a search for an unrelated donor can be initiated. The search for a healthy volunteer unrelated donor is carried out through an international network of mostly national registries called BM Donors Worldwide. Once the donor has been selected, various graft sources are available, including peripheral blood stem cells (PBSC), BM (BM) and placental cord blood (CB from voluntary donations). PBSCs are often chosen over BM because they are technically easier to collect and have a higher content of HSCs and T lymphocytes. In addition, compared with a stem cell transplant obtained from BM or CB, a transplant using PBSC offers the advantages of faster hematopoietic and the immune recoveries in the recipient, and a lower rate of graft failure. However, these advantages are offset by a higher incidence of graft-versus-host disease (GVHD), whereby donor immune cells react against the recipient cells, leading to multiple

organ damages. To prevent graft rejection and GVHD, patients receive immunosuppressive agents for a period of months.

### 2.1.1 Conditioning, aplasia and post-engraftment period

Allo-HSCT generally begins with a short period of chemotherapy and/or total body irradiation called conditioning. This conditioning aims to destroy the recipient's BM, including the cancer cells. It also suppresses the patient's immune system and induces sufficient immunosuppression to allow the donor HSCs to engraft. In addition, donor immune cells will exert a non-specific immune action against recipient tumor cells known as graft-versus-tumor effects, essential for ongoing disease control. Allogeneic transplantation therefore exerts myeloablative, immunosuppressive and antitumoral effects. The intensity of conditioning regimens may range from the most intensive myeloablative (MA) to less intense reduced-intensity (RIC) or even non-myeloablative (NMA) conditioning, chosen based on the recipient's disease status, age and physical condition. The distinction between these different regimens is based on criteria that encompass factors such as host hematopoiesis, hematologic recovery timeline, and the presence of mixed chimerism upon engraftment (453). RIC regimens combine moderate doses of immunosuppressive agents like fludarabine with NMA doses of alkylating agents to achieve substantial anti-tumor effects and control malignancy before graft-related immune effects against the tumor emerge (454, 455). In contrast, NMA conditioning relies on potent immunosuppression to facilitate engraftment of donor hematopoietic and immune cells, primarily eradicating both host hematopoiesis and tumor cells through graft-versus-host immune effects (456).

Once conditioning is completed and chemotherapy agents cleared from the recipient’s blood, the graft is infused intravenously. Recipients also benefit from prophylaxis against various infectious (bacterial, fungal, viral or parasitic infections) (457) and non-infectious complications, including hepatic sinusoidal obstruction syndrome (458) and GVHD (459).

### 2.1.2 Outcomes after allogeneic HSC transplantation

The growing number of allo-HSCT is attributable to multiple factors, including improved donor availability (unrelated and haploidentical donors) (445), RIC regimens (460), advances in the prevention and treatment of toxicities (infections and GVHD)(461, 462) and improved general supportive care. Despite these advances, allo-HSCT remains associated with significantly high rates of mortality, relapse, and complications (463).

Indeed, **long-term survival** after allo-HSCT has improved in recent years but ranges from 10% to 90% depending on the patient populations (464-468). The most frequent cause of mortality after allo-HSCT, both early and late, is **relapse of the initial disease** (464, 465). This is followed by transplant-related complications, notably infections and GVHD, leading to non-relapse mortality

(NRM)(465, 468, 469). Survival is associated with several factors, including patient age and comorbidities, disease characteristics (type of disease and cytogenetic risk) and status at transplantation (complete remission or not, and positive or negative minimal residual disease (MRD)), type of conditioning and source of hematopoietic stem cells, donor age and sex, HLA compatibility and cytomegalovirus (CMV) status (467, 470, 471). Moreover, the presence of moderate/severe cGVHD is a risk factor for mortality due to prolonged immunosuppression with a higher risk of infection, while limited cGVHD has been associated in some studies with better survival due to stronger GvL effects (472). In clinical practice, several prognostic scoring systems for survival after allo-HSCT are used to predict transplant outcomes more accurately before making decisions about transplant eligibility. These scores include, among others, the Disease Risk Index (DRI) (473-475), the Hematopoietic Cell Transplantation-Comorbidity Index (HCT-CI) (476) and the European Group for Blood and Marrow Transplantation (EBMT) risk score (477-479). The DRI is a powerful prognostic indicator of overall survival (OS) but does not consider patient comorbidities nor transplant characteristics (473, 474). On the contrary, the HCT-CI score (**Table 3**) only considers variables related to recipient comorbidities (476). While several donor and transplant factors are considered in the EBMT risk score, including donor type, sex and CMV matching, this score is based on old registry data that may not be that relevant nowadays.

**Relapse** is the leading cause of death after transplantation, but its incidence decreases with time elapsed since the transplant (464-467). For instance, Wong et al. reported disease relapse as the primary cause of early but not late mortality (50% of deaths in the overall cohort but only 10% among 15-year survivors) (464). Among 10,632 patients alive and disease-free 2 years after receiving MA allo-HSCT for hematological malignancies, late relapses remained the leading cause of death (27% to 42% of all deaths) (465), a finding also reported in other series (466, 467, 480-483). Many studies have shown that the greatest risk factors for relapse are incomplete response (including measurable residual disease) or relapse before transplantation, as well as high-risk cytogenetics in certain diseases. The number of such high-risk transplants (with high DRI) has significantly increased in recent years. Several studies have also demonstrated lower survival rates among patients with early (<6 months) compared to late relapse after allo-HSCT (471, 484). High-risk patients as well as those showing early signs of relapse after transplantation may benefit from preventive or preemptive strategies ranging from drugs aiming at targetable mutations to rapid infusion of donor lymphocytes (DLI) (485, 486).

**NRM** comprises all other causes of death besides persistence or relapse of the disease for which the transplant was performed. NRM decreases among patients transplanted in more recent years, illustrating improvements in preventive and curative treatments (464). However, NRM is not restricted to the early posttransplant period and the emergence of secondary neoplasms and

cardiopulmonary diseases is increasingly responsible for late non-disease-related deaths (464, 466, 467).

***Table 3*: Hematopoietic Cell Transplantation-Comorbidity Index score.** *ULN, upper limit of normal; ASAT, aspartate aminotransferase; ALAT, alanine aminotransferase; BMI, body mass index; SLE, systemic lupus erythematosus; RA, rheumatoid arthritis; CTD, connective tissue disease; DLCO, diffusion capacity of carbon monoxide; and FEV1, forced expiratory volume in one second.* ***Table adapted from Sorror et al (476).***

| Comorbidities | Definitions | HCT-CI weighted scores |
|---|---|---|
| **Arrhythmia** | Atrial fibrillation or flutter, sick sinus syndrome, or ventricular arrhythmia | 1 |
| **Cardiac** | Coronary artery disease, congestive heart failure, myocardial infarction, or ejection fraction<50% | 1 |
| **Inflammatory bowel disease** | Crohn's disease or ulcerative colitis | 1 |
| **Diabetes** | Requiring treatment with insulin or oral hypoglycemic, but not controlled with diet alone | 1 |
| **Cerebrovascular disease** | Transient ischemic attacks or cerebrovascular accident | 1 |
| **Psychiatric disturbance** | Depression/anxiety requiring psychiatric consult and/or treatment at the time of HCT | 1 |
| **Hepatic, mild** | Chronic hepatitis, bilirubin >ULN to 1.5× ULN, or ASAT/ALAT >ULN to 2.5× ULN | 1 |
| **Obesity** | Patients with a BMI of >35 for adults or with BMI-for-age percentile of ≥ 95th percentile for children | 1 |
| **Infection** | Documented infection or fever of unknown etiology requiring antimicrobial treatment before, during, and after the start of conditioning regimen | 1 |
| **Rheumatologic** | SLE, RA, polymyositis, mixed CTD, and polymyalgia rheumatica | 2 |
| **Peptic ulcer** | Requiring treatment | 2 |
| **Moderate/severe renal** | Serum creatinine > 2 mg/dL; on dialysis or prior to renal transplantation | 2 |
| **Moderate pulmonary** | DLco and/or $FEV_1$ 66%-80% or dyspnea on slight activity | 2 |
| **Prior solid malignancy** | Treated at any time point in the patient's history, excluding nonmelanoma skin cancer | 3 |
| **Heart valve disease** | Except asymptomatic mitral valve prolapse | 3 |
| **Severe pulmonary** | DLco and/or $FEV_1$ ≤65% or dyspnea at rest or requiring oxygen | 3 |
| **Moderate/severe hepatic** | Liver cirrhosis, bilirubin >1.5× ULN, or AST/ALT >2.5× ULN | 3 |

**Conditioning-related toxicities** can occur in the cutaneous, mucosal, gastrointestinal, pulmonary, cardiac, urinary, endothelial, or neurological systems. Hepatic veno-occlusive disease

(VOD), also known as sinusoidal obstruction syndrome (SOS), is a serious complication that can occur mainly after MA conditioning, but cases after RIC have also been reported (487). A meta-analysis of 135 studies reported an overall incidence of VOD of 13.7%, with a mortality rate of 84.3% in severe cases (487).

**Infections** are the most frequent complications because of immune suppression and prolonged neutropenia. During the early, pre-engraftment, posttransplant period, they are mainly associated with gram-positive and gram-negative bacteria, herpes simplex virus and CMV, candidiasis, and invasive aspergillosis (457). During the intermediate posttransplant period (from neutrophil recovery to day 100 posttransplant), despite recovery from neutropenia, cellular and humoral immunity remains impaired, and there is a risk of opportunistic infections, such as aspergillosis, Pneumocystis Jirovecii and CMV, and increased susceptibility to common respiratory viruses such as influenza, respiratory syncytial virus, and adenovirus (488, 489). Moreover, the presence of GVHD and its treatment entail a greater degree of immunosuppression. Consequently, recipients with active GVHD are at higher risk of invasive fungal infections and viral reactivation (490). Indeed, invasive fungal pneumonia has become increasingly common after allo-HSCT due to prolonged neutropenia and immunosuppressive therapies and the widespread use of broad-spectrum antibiotics (490, 491). The risk of late infections is also present. Indeed, after allo-HSCT and particularly after MA conditioning, immune reconstitution remains incomplete, which results in partial responses to vaccination and an increased risk of late opportunistic infections, particularly varicella zoster virus reactivations (472). However, bacterial infections remain the predominant cause of late infection-related mortality.

**GVHD** is an alloimmune disease caused by the recognition of recipient antigens by immunocompetent donor cells. This frequent complication is the source of significant morbidity and mortality. Prevention of GVHD is essential, and most often involves combining a calcineurin inhibitor such as cyclosporine or tacrolimus with an antimetabolite such as MTX or mycophenolate mofetil (MMF). The administration of anti-thymocyte globulins as part of allo-HSCT conditioning also contributes to the prevention of acute GvHD (492, 493). The mechanism of GVHD and the clinical presentation differ according to whether it is acute or chronic form. In 2005, the National Institute of Health consensus conference defined acute and chronic GvHD based on the type of clinical manifestations, rather than the timing of their onset relative to transplantation (494). This 2005 consensus was updated in 2014, introducing new criteria to diagnose and assess the severity of chronic GVHD (495). The 2014 NIH consensus includes a clarification of the overlap GVHD subcategory, adjustments to diagnostic criteria for each organ, and a revision of organ-specific severity scoring. Mild cases can be managed through observation or local treatments, while more severe cases require systemic treatment. Optimal management aims to use the least amount of treatment necessary until immunological tolerance develops.

**Acute GVHD** (aGVHD) affects around 30-50% of patients and generally occurs in the first few weeks after transplantation. The broad category of acute GVHD encompasses (1) classic acute GVHD occurring within 100 days after transplantation or DLI in a patient not meeting criteria for the diagnosis of chronic GVHD and (2) persistent, recurrent, or late onset acute GVHD with features of classic acute GVHD occurring beyond 100 days post transplantation or DLI in a patient not meeting criteria for the diagnosis of chronic GVHD (495). The most affected organs are the skin, digestive tract, and liver (496). The severity of aGVHD ranges from grade I for the mildest forms to grade IV for the most severe (497). Severe aGVHD is associated with poorer survival (498). Systemic corticosteroids are the primary treatment for aGVHD (499). They have anti-inflammatory and immunosuppressive effects, which help suppress the immune response and control the GVHD. However, some cases of GVHD do not respond well to corticosteroids and are considered steroid-refractory. Available options for steroid-resistant aGVHD encompass anti-thymocyte globulins, various monoclonal antibodies (alemtuzumab, basiliximab, daclizumab, vedolizumab…), mTOR inhibitors (sirolimus, everolimus…), JAK inhibitors (ruxolitinib…), MMF, MTX, pentostatin, α1-antitrypsin, extracorporeal photopheresis, fecal microbiota transplantation, and cellular therapies such as mesenchymal stromal cells (MSC) (499), but only ruxolitinib has proven to be superior to best available therapies in this setting (500).

**Chronic GvHD** (cGVHD) affects 40-70% of patients after allo-HSCT and is the main medium- and short-term complication (501). Chronic GVHD shares similarities with autoimmune diseases, and the most frequently observed clinical manifestations of chronic GvHD are cutaneous (65-80%), oral (48-72%), hepatic (40-73%) and ocular (18-47%) disorders. The median onset is 6 months posttransplant, with most cases occurring within 2 years posttransplant (502). Although its prevalence declines after 2-3 years, nearly 20% of patients with cGVHD require immunosuppressive therapy for up to 5 years posttransplant (503). In addition, many patients present sequelae of chronic GvHD that impair quality of life (dry eye syndrome, bronchiolitis obliterans, etc.). Steroids are the primary therapy for chronic GVHD and second-line therapies in steroid-refractory, -dependent or -intolerant patients include many of the treatments already mentioned for aGVHD (499, 504). However, here also ruxolitinib is the only drug that has demonstrated efficacy compared to best available treatment in such patients (505). Nevertheless, inhibition of the wild-type JAK2 protein can lead to myelosuppression, primarily characterized by anemia and thrombocytopenia and less frequently by neutropenia, possibly leading to complications following allogeneic transplantation (506, 507).

After HSCT, **hematopoietic recovery** occurs after engrafted HSCs proliferate and differentiate to produce donor-derived mature blood cells. Granulocytes and platelets recover first, whereas complete erythrocyte engraftment takes more time (508). Neutrophil engraftment, defined as the first occurrence of three consecutive days with a neutrophil count exceeding $0.5\times10^9$/L, typically

falls within 10 to 21 days when using PBSC, up to 30 days when using BM, and extending to 42 days when utilizing umbilical cord blood (UCB) (509). Platelet engraftment, the first of three consecutive days with a platelet count of 20,000/µL without transfusion for seven consecutive days (509), ranges between 15 and 30 days with PBSC or BM, but may take up to 100 days with UCB (509). RBC engraftment typically occurs within a variable timeframe of 3 to 12 months (509). This variability depends on the type of conditioning (MA, RIC, or NMA) (510), the dose and source of hematopoietic stem cells (511, 512), and whether growth factors such as G-CSF and/or ESAs are prescribed. During engraftment, the patient will typically receive multiple RBC and/or platelet transfusions, but the need for transfusions is substantially reduced after non-myeloablative or RIC compared to myeloablative conditioning, although it is also influenced by pre-transplantation platelet counts and hemoglobin levels, the source and dose of hematopoietic stem cells, minor and major ABO mismatch, as well as the occurrence and severity of acute GVHD and other complications (513).

## 2.2 Erythropoiesis and iron during allogeneic HSC transplantation

### 2.2.1 Erythropoiesis

#### *2.2.1.1 Erythropoietic recovery and EPO production*

After HSCT, complete recovery of erythrocytes is delayed. Several mechanisms are involved, such as delayed engraftment, BM toxicity induced by chemo-radiotherapy, cytotoxic drugs, and the production of inhibitory cytokines (514). However, one of the most incriminating factors is defective erythropoietin production (515). Indeed, variations in EPO production during allo-HSCT are well described: after allo-HSCT conditioning, serum EPO levels rise rapidly (516, 517). Then, EPO levels decrease during engraftment, and this is even followed by EPO deficiency for 1 to 3 weeks thereafter (516).

***After conditioning***, serum EPO levels increase transiently, peaking between days 0 and 7 post-transplant when erythropoietic activity is at its lowest (518, 519). This increase occurs even in the absence of Hb variations, whether in the context of autologous HSCT (auto-HSCT) or allo-HSCT and does not depend on the type of graft (BM or PBSC) or type of conditioning (with or without TBI). Indeed, the rate of EPO utilization by erythroid precursors can lead to variations in serum EPO levels (520, 521). Consequently, the EPO peak during conditioning-induced myelosuppression is secondary to reduced EPO consumption. Decreased EPO clearance because of marrow aplasia therefore prolongs EPO lifespan. This involvement of the BM in EPO clearance has been confirmed in animal models, where a progressive decrease in EPO clearance after myelosuppressive treatment has been observed (522).

***During engraftment***, BM erythroid precursors recover and proliferate, consuming EPO and leading to a progressive decrease of EPO levels to the normal range (516). The speed of

engraftment is inversely correlated with the duration of this correction phase; thus, insufficient EPO levels for the degree of anemia are sometimes observed in patients whose erythropoiesis is very active during marrow recovery. Our group has confirmed this hypothesis by showing that EPO production is inversely correlated with erythropoietic activity (515). Indirect observations in EPO-treated or hypoxia-exposed mice suggest that increased erythropoiesis may also depress endogenous EPO production by unknown mechanisms (523).

***After erythropoietic recovery***, serum EPO levels evolve differently depending on the type of transplantation (autologous or allogeneic) (516, 519, 524). After auto-HSCT, serum EPO levels remain adequate for the degree of anemia (516, 525), while they are inadequately low after allo-HSCT. Hence, after auto-HSCT, the recovery of erythropoiesis is more limited by the insufficient number of erythroid precursors (517) than by insufficient EPO production, even if EPO levels may be transiently inadequate (517, 526). On the other hand, after MA allo-HSCT, EPO levels increase in absolute terms but not sufficiently for the degree of anemia, resulting in inadequate EPO production and prolonged anemia (516). In contrast, following NMA allo-HSCT, serum EPO levels remain adequate throughout the posttransplant period (527).

Finally, EPO production during allo-HSCT is also negatively influenced by various other factors, including drugs and conditioning-induced cytokines.

Cyclosporine or tacrolimus can impair renal function and EPO production. Indeed, EPO deficiency is more severe if GVHD prophylaxis consists in cyclosporine rather than T-cell depletion (528), and the degree of EPO insufficiency correlates with the dose of cyclosporine (515, 516, 518). Some studies have shown that this is even independent of renal function (518, 525). However, after NMA conditioning, EPO levels remain adequate, even in recipients with elevated cyclosporine blood concentrations (527). Importantly, EPO production is not influenced by azathioprine or mycophenolate mofetil (527, 529), two other immunosuppressive drugs used after allo-HSCT, but these two agents can induce EPO resistance in renal transplant recipients (529).

Acute GVHD is another factor aggravating EPO deficiency (515, 516, 524), but this has not been observed in all studies (518). In contrast, cGVHD had no effect on EPO production (515, 516, 524). After NMA conditioning, aGVHD is less frequent (527) than after MA conditioning, suggesting that this may contribute to maintaining adequate EPO levels after NMA conditioning.

CMV reactivation or infection are also associated with decreased serum EPO levels (515, 518), whereas CMV-positive status per se does not result in decreased EPO production (524). However, as CMV reactivation rates are similar after NMA or MA conditioning (527), this cannot explain the difference in EPO levels between patients undergoing MA or NMA allo-HSCT.

Lastly, many cytokines produced in excess during GVHD (530) or CMV infection (531) may also influence serum EPO levels. Indeed, as mentioned above, IL-6 stimulates EPO production, while IL-1, TNF-α, IFN-γ and TGF-β inhibit it (532). Holler et al have shown that increased serum levels

of TNF-a precede major complications of HSCT (such as CMV reactivation, infection or GVHD) and can suppress EPO production (533).

#### *2.2.1.2 Erythropoiesis-stimulating agents after transplantation*

To date, there are only two treatments for anemia after allogeneic HSCT: RBC transfusions and the use of ESAs (534).

The efficacy of rHuEPO in stimulating RBC production has also led to its use in HSCT to help accelerate hematopoietic recovery after transplantation (535-537). Initially, rHuEPO was surprisingly shown to be effective after allogeneic HSCT in patients suspected of pure red cell aplasia (PRCA) (538) of alloimmune origin in ABO incompatibilities (539, 540). The source of auto- or allo-immunity may be donor- or recipient-related, including passive antibody transfusion, passenger lymphocyte syndrome, alloantibodies formed against transplanted hematopoietic cells, or new auto- or allo-antibodies resulting from transfusions (538). Approximately 30-50% of allo-HSCT are performed with ABO blood group minor and/or major incompatibility. This ABO incompatibility can lead to serious clinical complications, including acute hemolysis in 10-15% of cases (538), and pure red cell aplasia (540, 541). The efficacy of rHuEPO in the setting of PRCA could be that when erythrocyte production exceeds a certain agglutinin threshold, the agglutinin titer would decrease by absorption and increased erythropoiesis would overcome residual inhibition. However, subsequent studies have not demonstrated the efficacy of rHuEPO in PRCA (542, 543).

However, as mentioned above, one of the main mechanisms of anemia after allogeneic HSCT is a pronounced deficit of EPO production in response to anemia (515, 516, 544), except after nonmyeloablative HSCT (527). After autologous HSCT, the limiting factor for erythroid regeneration is above all the relative paucity of hematopoietic progenitors (517). It was therefore not surprising to find out that rHuEPO treatment immediately after transplantation did not accelerate the recovery of the red blood lineage after auto-HSCT but showed some evidence of efficacy after allo-HSCT (126). Subsequently, it was shown that the use of rHuEPO was highly effective after auto-HSCT (526) or allo-HSCT (545, 546), provided that it is started after initial engraftment. The use of rHuEPO accelerates erythroid recovery and reduces transfusion requirements after both MA and NMA (547), without altering the risk of relapse or survival (548). After NMA, starting rHuEpo administration as soon as from day 0 posttransplant significantly reduced the need for RBC transfusions in the first month after transplantation (549). Higher levels of donor T-cell chimerism were identified as the strongest predictor for achieving Hb values of 13 g/dl, suggesting the potential inhibitory effect of residual recipient lymphocytes on donor erythropoiesis (513, 549). Subsequently, our group observed a poorer response to rHuEPO in patients with aGVHD, while cGVHD did not appear to affect EPO response (515). Finally, the

addition of IV iron supplementation, as now recommended by published guidelines on EPO therapy in cancer patients (149), increased response rates and reduced the number of rHuEPO doses required to achieve a complete response to ESAs (550) without significant short- or long-term toxicity (548, 551).

### 2.2.2 Iron metabolism

Over the past decade, hyperferritinemia before and after allogeneic HSCT has been extensively reported (552-557). These high SF levels have been initially considered as a marker of IO in most studies. Already before conditioning, many patients have elevated SF levels (557-565). The most common cause of elevated SF levels before and after transplantation is thought to be the transfusion of multiple RBC units during conventional treatment of hematologic malignancies or severe non-malignant disorders involving the red cell lineage, as IO can already be observed after transfusion of only 10 to 20 RBC units (417). After conditioning, ferritin usually peaks in the first few months after transplantation and declines very slowly thereafter (565-569). In addition, elevated NTBI has been reported in several studies and a number of mechanisms have been invoked to explain these observations, as described in **Table 4** (570).

***Table 4*: Causes of increased NTBI in HSCT.** *Table adapted from Pullarkat et al (570).*

| Timing relative to transplantation | Source of iron | Underlying mechanism |
|---|---|---|
| **Before and after** | Increased macrophage iron | Red cell transfusion therapy |
| **Before** | Increased intestinal iron absorption due to low hepcidin | Feature of some chronic anemias (e.g., MDS, thalassemia intermedia) |
| **During conditioning** | Release of cellular iron | Destruction of bone marrow and tumor cells as a result of cytotoxic therapy used as part of the conditioning regimen |
| | Underutilization of plasma iron | Inhibition of hepatocytes decreases transferrin production |
| **After conditioning, during aplasia** | Underutilization of plasma iron | Inhibition of erythropoiesis because of cytotoxic therapy used as part of the conditioning regimen |
| | | Inhibition of hepatocytes decreases transferrin production |

***Prior to transplantation***, as mentioned above, the primary cause of IO-related high SF is the number of transfusions. Another mechanism is ineffective erythropoiesis. Indeed, as mentioned under 1.2.3.2 “iron overload”, ineffective erythropoiesis, as encountered in MDS, induces the

secretion of erythroferrone, which inhibits hepcidin production and thus increases iron absorption by the gut and iron release by macrophages (423). This contributes to increase NTBI, a toxic form of SeFe then taken up by parenchymal tissues through a TfR-independent mechanism.
***During conditioning,*** the cytotoxic action of chemotherapy can lead to iron release from dying RBCs and hepatocytes, further increasing NTBI (569, 571). Naoum *et al* demonstrated that NTBI levels, albeit normal at baseline, increased substantially 48 hours after the start of conditioning in HSCT patients, with a peak around Day 0, and remained increased until engraftment, when they returned to normal levels (572).
***After conditioning,*** suppression of erythropoiesis also contributes to this IO, resulting in decreased marrow demand for iron, **increased transferrin saturation** because of non-utilization of SeFe, and further iron deposition in tissues. Indeed, our group has demonstrated that serum sTfR levels (quantitative marker of erythropoietic activity) decreased by more than 50% after conditioning in allo-HSCT, before gradually returning to adequate levels thereafter (517). Consequently, this contributes to IO as the SeFe pool is not utilized during aplasia (573). Similarly, after conditioning, Tsat is very high and corrects only with the resumption of erythropoiesis (569, 574, 575); this also points to the non-utilization of iron by the BM. Finally, hepatocytes, destroyed by chemotherapy, are unable to produce the transferrin that should transport SeFe, which also contributes to an increase in the free iron pool (557). In this early phase, the pro-oxidant properties of **NTBI** can exacerbate toxic complications (405).
Normally, endogenous antioxidants help scavenge free radicals and prevent cellular damage (576). However, it has been shown that during allo-HSCT there is a loss of these natural antioxidant mechanisms (a measure of the overall capacity of human plasma to inhibit free radical-induced lipid peroxidation) in favor of a pro-oxidant status (575, 577). The imbalance of the pro-oxidant/antioxidant ratio in the plasma of patients undergoing allo-HSCT can therefore participate to increase NTBI toxicity (575).
However, several studies (**Table 5**) have highlighted (in addition to the obvious relation with the number of transfusions) a correlation between elevated **pretransplant SF levels** and CRP, IL-6 and hepcidin. Some studies have also shown that pre-transplant ferritin levels are significantly higher with low serum albumin, in AML patients, when International Prognostic Scoring System (IPPS), DRI and HCT-CI scores are poorer, when the number of previous treatment lines is higher and when the donor is unrelated. This highlights that, in addition to the mechanisms leading to IO during allo-HSCT, serum ferritin may be elevated due to inflammation secondary to severe malignant diseases, recipient comorbidities, multiple chemotherapies or infections, and is not solely a reflection of systemic IO.
As mentioned above, animal models of inflammation (385) and thalassemia (386) have demonstrated that the negative impact of erythropoietic activity dominates the positive effect of

inflammation or IO in **hepcidin** regulation. After autologous HSCT, our group observed that the main determinants of hepcidin production were iron stores and erythropoietic activity, while inflammation played a minor role, confirming the findings in animal models (387). In allogeneic HSCT, few studies have looked at the predictors of hepcidin levels during allogeneic transplantation. **Table 5** also lists the studies that measured serum hepcidin values during allo-HSCT. All studies showed relatively high pretransplant hepcidin levels compared with normal individuals. The largest cohort is that of Sakamoto et al. on 166 patients who underwent MA and RIC allo-HSCT for acute leukemia (578). Median pretransplant hepcidin levels were 35 ng/mL and positively correlated with pretransplant ferritin levels ($r=0.57$, $p< 0.001$). Curiously, Aki et al did not demonstrate such a correlation between pretransplant ferritin and hepcidin or between pretransplant hepcidin and Il-6, whereas there was a positive correlation between pretransplant ferritin and IL-6 (560). Armand et al, in addition to observing a positive correlation between pretransplant hepcidin and ferritin ($r=0.70$), showed a correlation between pretransplant hepcidin and previous RBC transfusions ($r=0.65$), as well as, to a lesser extent, between hepcidin and liver iron concentrations (measured by MRI) ($r=0.53$) or with C-reactive protein (CRP) ($r=0.39$) (579). Kanda also compared patient characteristics between two groups separated on the basis of pretransplant hepcidin values: no differences were observed according to recipient age or gender, type of disease, DRI, type of conditioning, GVHD prophylaxis, type of donor, or source of HSCs (580). Similarly, Eisfeld et al reported no correlation with recipient age or gender, liver or kidney function, number of prior chemotherapies and type of conditioning (557). However, they showed that AML patients had very high serum hepcidin levels before and after transplantation compared those in the control group ($p<0.0001$), and that pre- and post-transplant hepcidin levels were correlated with the number of pre- and post-transplant RBC transfusions ($p<0.001$), as well as with low pre- and post-transplant Hb levels ($p=0.02$). Curiously, no correlation was found between pretransplant ferritin and hepcidin levels.

**Table 5** summarizes the studies that investigated factors affecting pre-HSCT serum ferritin and hepcidin values. None of them considered the full range of patient characteristics, disease, transplant and donor characteristics.

***Table 5*: Summary of studies analyzing the factors influencing pretransplant serum ferritin and hepcidin in allogeneic HSCT**. *AL: acute leukemia; AML: acute myeloid leukemia; AMLs: secondary acute myeloid leukemia; ALAT: alanine aminotransferase; allo-HSCT: allogeneic hematopoietic stem cell transplantation; auto-HSCT: autologous hematopoietic stem cell transplantation; BEAM: Carmustine, Etoposide, Cytarabine, Melphalan; CML: chronic myeloid leukemia; CR: complete remission; CRP: C-reactive protein; DRI: disease risk index; EBMT: European Group for Blood and Marrow Transplantation; ESR: erythrocyte sedimentation rate; a/c GVHD: acute/chronic graft-versus-host disease; Hb: hemoglobin; HCT-CI score: Hematopoietic Cell Transplantation-Comorbidity Index; IFI: invasive fungal infection; IL-6: interleukin-6 ; IPSS: International Prognostic Scoring System; LIC: liver iron concentration; M: mean; MA: myeloablative; MDS: myelodysplastic syndrome; MM: multiple myeloma; MUD: matched unrelated donor; MV: multivariate analysis; N: number; NMA: nonmyeloablative; NR: not reported; NS: no significant; postT: posttransplant; preT: pretransplant; RBC: red blood cells; RIC: reduced-intensity conditioning; sTfR: transferrin soluble receptor; SF: serum ferritin; SD: standard deviation; Tsat: transferrin saturation; Tx: transfusion; UD: unrelated donor; UV: univariate analysis; y: year.*

| Author, year | Characteristics of the cohort | | | | | Pretransplant serum ferritin | Pretransplant serum hepcidin |
|---|---|---|---|---|---|---|---|
| | Type of study | N patients | Disease | Transplant & conditioning | Pretransplant serum ferritin & hepcidin | Associated factors | Associated factors |
| ***Kanda, 2009*** | Retrospective | 55 | Hematologic malignancies | Allo-HSCT MA, RIC | PreT SF: median NR<br>PreT hepcidin: median 21.6 ng/mL (1.4–371) | High preT hepcidin ➔ high SF *(UV; MV not done)* | High preT SF ➔ high hepcidin *(UV; MV not done)*<br>Reticulocytes, CRP, age, sex, disease, DRI ➔ NS effect |
| ***Aki, 2011*** | Retrospective | 100 | Hematologic malignancies | Allo-HSCT MA, NMA | PreT SF: M±SD 1,222 ± 1,304 ng/mL.<br>PreT hepcidin: M±SD 137.1 ± 54.7 ng/mL | High preT CRP or ESR or IL-6 ➔ high SF *(UV; MV not done)*<br>High preT hepcidin ➔ NS effect on SF | PreT SF or IL-6 ➔ NS effect on hepcidin |
| ***Armand, 2011*** | Prospective | 48 | AL, MDS | Allo-HSCT MA | PreT SF: median 1,549 ng/mL (20–6,989)<br>PreT hepcidin: median 59 ng/mL (10–468) | High preT hepcidin or MRI LIC ➔high SF *(UV; MV not done)* | High preT SF or MRI LIC or CRP or RBC Tx ➔ high hepcidin *(UV; MV not done)* |
| ***Eisfeld, 2011*** | Prospective | 42 | AML | Allo-HSCT MA, RIC | PreT SF: median 1,945 ng/mL (617–6,981)<br>PreT hepcidin: median 358 ng/mL (56–1,096) | High preT RBC Tx, low preT Hb ➔ high SF *(UV; MV not done)*<br>High preT hepcidin ➔ NS effect on SF | High preT RBC Tx, low preT Hb ➔ high hepcidin *(UV; MV not done)*<br>PreT SF, age, sex, liver or renal function, N treatment lines ➔ NS effect on hepcidin |
| ***Sakamoto, 2017*** | Retrospective | 166 | Hematologic malignancies | Allo-HSCT MA, RIC | PreT SF: median 694 ng/mL<br>PreT hepcidin: median 35 ng/mL | High preT hepcidin ➔ high SF *(UV; MV not done)* | High preT SF ➔ high hepcidin *(UV; MV not done)* |
| ***Altes, 2002*** | Prospective | 25 | AL, CML, lymphoma | Auto & allo-HSCT MA, BEAM | PreT SF: median NR | High preT RBC Tx ➔ high SF *(UV; MV not done)*<br>High preT Tsat ➔ NS effect on SF | |
| ***Kim, 2008*** | Retrospective | 38 | Hematologic malignancies | Allo-HSCT RIC | PreT SF: median in<br>- low SF (<1,000 ng/mL) group: 739 ng/mL<br>- high SF (>1,000 ng/mL) group: 1,578 ng/mL | High preT RBC Tx ➔ high SF *(UV; MV not done)* | |
| ***Platzbecker, 2008*** | Retrospective | 172 | AL, MDS | Allo-HSCT MA | PreT SF: median 924 µg/L (27–6,500) | Tx dependence, high HCT-CI ➔ high SF *(UV; MV not done)* | |
| ***Mahindra, 2009b*** | Retrospective | 222 | AL, CML, MDS, NHL | Allo-HSCT MA | PreT SF: median 1,433 ng/mL (7–17,029) | AL, N treatment lines ➔ high SF *(UV; MV not done)* | |
| ***Alessandrino, 2010*** | Retrospective | 129 | MDS | Allo-HSCT MA, RIC | PreT SF: median 1,270 ng/mL | High preT RBC Tx ➔ high SF *(UV; MV not done)* | |
| ***Lim, 2010*** | Retrospective | 99 | MDS, AML | Allo-HSCT RIC | PreT SF: median 1,992 ng/mL (6–9,580) | AML, high IPSS, N treatment lines, >5% blasts ➔ high SF *(UV; MV not done)* | |

| Author, year | Characteristics of the cohort | | | | | Pretransplant serum ferritin | Pretransplant serum hepcidin |
|---|---|---|---|---|---|---|---|
| | Type of study | N patients | Disease | Transplant & conditioning | Pretransplant serum ferritin & hepcidin | Associated factors | Associated factors |
| ***Sucak, 2010*** | Retrospective | 250 | Malignant & nonmalignant hematologic disorders | Allo & auto-HSCT MA, RIC | PreT SF: median 1,403 ng/mL | Allo: high HCT-CI or PS ➔ high SF *(UV; MV not done)*<br>Auto: high PS ➔ high SF *(UV; MV not done)* | |
| ***Tachibana, 2011a*** | Retrospective | 143 | AL | Allo-HSCT MA, RIC | PreT SF: median 1,200 ng/ml (AML) & 960 ng/mL (ALL) | AML & ALL: high DRI ➔ high SF *(UV; MV not done)* | |
| ***Kanda, 2011*** | Retrospective | 112 | Hematologic malignancies | Allo-HSCT MA, RIC | PreT SF: median 695 ng/mL | High preT CRP ➔ NS effect on SF | |
| ***Wahlin, 2011*** | Retrospective | 309 | Hematologic malignancies | Allo-HSCT MA, RIC | PreT SF: median 1,159 ng/mL | AML/MDS, high preT ALAT or CRP ➔ high SF *(UV; MV not done)* | |
| ***Aki, 2012*** | Retrospective | 106 | AL | Allo-HSCT MA | PreT SF: median 1,337 ng/mL | High preT CRP, HCT-CI, previous IFI ➔ high SF *(UV; MV not done)*<br>EBMT risk score ➔ NS effect on SF | |
| ***Bazuaye, 2012*** | Retrospective | 230 | Malignant & nonmalignant hematologic disorders | Allo-HSCT MA | PreT SF: median 1,342 ng/mL. | Low preT sTfR, high preT SeFe or Tsat, high preT RBC Tx, AML (vs other diagnoses), male ➔ high SF *(UV; MV not done)*<br>High preT CRP ➔ NS effect on SF | |
| ***Sivgin, 2012*** | Retrospective | 84 | Malignant & nonmalignant hematologic disorders | Allo-HSCT MA, NMA | PreT SF: median in<br>- low SF (<1,000 ng/mL) group: 389 ng/mL.<br>- high SF (>1,000 ng/mL) group: 1,705 ng/mL | High preT SeFe or Tsat ➔ high SF *(UV; MV not done)*<br>High preT albumin ➔ NS effect on SF | |
| ***Tachibana, 2012*** | Retrospective | 261 | AL, MDS | Allo-HSCT MA, RIC | PreT SF: median 970 ng/mL | PreT disease status (not CR), AML ➔ high SF *(UV; MV not done)* | |
| ***Wermke, 2012*** | Retrospective | 88 | AML, MDS | Allo-HSCT MA, RIC | PreT SF: median 1,928 ng/ml (26-14,179) | High preT RBC Tx or Tsat or CRP, high HCT-CI ➔ high SF (*UV; MV not done)*<br>High MRI LIC (≥ 125 µmol/g) ➔ high SF | |
| ***Sakamoto, 2013*** | Retrospective | 211 | Malignant & nonmalignant hematologic disorders | Allo-HSCT MA, RIC | PreT SF: median 470 ng/mL | High preT CRP ➔ high SF *(UV; MV not done)* | |
| ***Tanaka, 2015*** | Prospective | 190 | AL, MDS | Allo-HSCT MA, RIC | PreT SF: median in<br>- low SF (<1,000 ng/mL) group: 541 ng/mL.<br>- high SF (>1,000 ng/mL) group: 1,610 ng/mL | Male sex, high preT RBC Tx ➔ high SF *(UV; MV not done)* | |
| ***Artz, 2016*** | Retrospective | 784 | AML, MDS | Allo-HSCT MA, RIC, NMA | PreT SF: median 1,148 ng/mL (51-14,298) | AML, high preT CRP, high HCT-CI➔ high SF (*UV; MV not done)*<br>Age ➔ NS effect on SF | |
| ***Fingrut, 2021*** | Retrospective | 229 | Malignant & nonmalignant hematologic disorders | Allo-HSCT MA, RIC | PreT SF: median 1,052 ng/mL (32-12,074) | AML, high PS, high HCT-CI ➔ high SF<br>Age, sex, DRI ➔ NS effect on SF | |

### 2.2.3 Prognostic impact of iron parameters on outcomes after allogeneic HSC transplantation

Table 6 summarizes all studies analyzing the impact of pretransplant serum ferritin (and/or hepcidin when mentioned) on OS, PFS and NRM after allo-HSCT, while table 7 presents its impact on various complications (conditioning-induced toxicities, infections and GVHD) and table 8 on hematologic recovery.

There is strong evidence suggesting that elevated pretransplant SF is associated with poor outcomes after allo-HSCT (578, 579, 581-584). Indeed, several studies demonstrated that elevated pretransplant SF was associated with **inferior OS** and **progression-free survival** (PFS), as well as higher **NRM** (Table 6). Yan *et al* published the largest meta-analysis including 25 studies investigating the prognostic impact of pretransplant SF on different outcomes in 4,545 patients undergoing allo-HSCT (585). The results showed that high pretransplant SF was significantly associated with lower overall OS and PFS (hazard ratio (HR) 1.5 and 1.72, respectively, $p<0.001$ for both) as well as a high incidence of NRM (HR 2.28, $p<0.001$). Moreover, Vaughn *et al.* demonstrated that the addition of three relevant biomarkers (SF > 2,500 ng/ml, albumin < 30 g/L, and platelets < 20,000/µL) to the HCT-CI score was independently associated with increased NRM and decreased OS (586).

However, most studies have shown no impact of ferritin on **disease relapse**, except for some reporting higher pretransplant SF (100 days to 2 weeks prior to HSCT) in AML patients who relapsed post-HSCT (559, 587-589). Then, only 2 studies have observed an impact of pretransplant SF on the risk of relapse in multivariate analyses. Jang et al observed that low pretransplant SF (<1,400 ng/mL), together with CR and favorable cytogenetics before HSCT, as well as early CMV replication and cGVHD after HSCT, decreased the risk of relapse in 74 AML patients (590). Sakamoto et al also demonstrated a negative effect of pretransplant SF on relapse in multivariate analysis (high pretransplant CRP and high-risk DRI were also found as risk factors for relapse) (591).

Only four studies investigated the impact of pretransplant hepcidin on outcomes after allo-HSCT (557, 560, 578, 580), and only Sakamoto et al (578) demonstrated a significantly negative impact of high pretransplant hepcidin (≥ 35 ng/mL) levels on OS in 166 patients (HR: 1.94, $p=0.007$). Indeed, at 3 years, patients in the high hepcidin group had lower OS than those with low hepcidin (49.2 vs. 69.0%, respectively, $p=0.006$), although no significant difference was observed in terms of NRM (14 vs 11 events) or disease relapse (30 vs 16 events). In multivariate analysis, high pretransplant serum hepcidin (HR 2.01, $p=0.005$), male gender (HR 1.92, $p=0.008$), and high DRI (HR 2.37, $p<0.001$) were independently associated with mortality (578). Similarly, Eisfeld et al did not show a significant influence of pretransplant hepcidin on NRM or relapse (557).

Several teams have studied the impact of pretransplant SF on **conditioning-related toxicities, and particularly on the risk of developing VOD** (Table 7) (560, 564, 592-596). Morado et al. were the first to demonstrate an association between elevated pretransplant SF levels and the development of VOD in patients after auto-HSCT (597) and subsequent publications reported the same association in allo-HSCT (560, 564, 592, 594). Furthermore, several studies based on hepatic biopsies have clarified that the primary cause of hepatic test abnormalities following allo-HSCT was IO, then correlating with elevated SF levels (598). However, alternative investigations failed to substantiate a significant correlation between pretransplant SF and the risk of VOD (593, 595, 596). Aki *et al* are the only ones who investigated the impact of high pretransplant hepcidin levels on various toxicities, finding an association with mucositis, but no significant effect on VOD or hepatic, cardiac, renal, and pulmonary toxicities (560).

Iron acquisition is essential for the growth and virulence of many pathogenic microorganisms, including Aspergillosis species (599). It is therefore likely that IO would increase the **incidence of infection** (Table 7) and NRM after allo-HSCT. Indeed, some studies have shown an association between high pretransplant SF levels and the risk of bacterial infections (mainly bloodstream infections) (581, 582, 600-605) but also of invasive fungal infections (564, 594, 605, 606). However, the extensive meta-analysis by Yan *et al*, involving 25 studies with 4,545 patients, did not demonstrate that pre-transplant serum ferritin contributed to an increased incidence of infections (OR 1.67, p=0.09) (585). Similarly, two studies reported that pretransplant serum hepcidin was associated with a higher infection rate in multivariate analysis (HR 28.46, p=0.007) (580) as well as with an increase in the number of days of febrile neutropenia (560). No significant association has been reported between SF (593) or hepcidin (580) and CMV reactivation.

Most studies have not shown a significant effect of pretransplant SF on the **risk of acute or chronic GVHD** (Table 7). Only 3 studies showed a positive association between pretransplant SF and the development of aGVHD (561, 607, 608), while 2 studies (558, 591) showed a negative association with the development of cGVHD and especially in AML (591). These observations were clarified by the meta-analysis of Yan *et al* who demonstrated a lower incidence of cGVHD with high pretransplant SF levels (OR=0.74, P<0.05) but no significant relationship with aGVHD (585). The impact of pretransplant hepcidin on the incidence of aGVHD was studied by Sakamoto *et al* and no significant effect was demonstrated (578). No study has examined the impact of pretransplant hepcidin on cGVHD.

As previously mentioned, pretransplant SF (557, 593) or hepcidin (557) levels are correlated with the number of previous RBC transfusions. These biomarkers have also been sometimes associated with slower or poorer hematological recovery after transplantation. Indeed, Aki *et al* and Tanaka *et al* showed that high pretransplant SF correlated with poorer neutrophil recovery (560, 563). Once again, results diverge as most other studies have not demonstrated a significant effect on

neutrophil, platelet or Hb recovery (578, 593, 602, 605, 609). Very few studies have investigated the impact of pretransplant SF on the number of transfusions after allo-HSCT. Aki *et al* showed a positive correlation between pretransplant SF and the number of RBC (r=0.26, p=0.01) and platelet (r=0.25, p=0.02) transfusions after allo-HSCT (560). Concerning pretransplant hepcidin, no significant impact on neutrophil (560, 578, 610) or Hb (578) recoveries, nor on posttransplant RBC or platelet transfusions (560), has been observed. A significant negative effect on platelet recovery has been reported in a single study in uni- as well as multi-variate analyses (HR 0.59, p=0.003) (578) but not in another one (560) . Other variables negatively influencing platelet recovery in a multivariate Cox model were high DRI (HR 0.65, p=0.02), use of unrelated cord blood grafts (HR 0.37, p<0.001) and male sex (HR 0.69, p=0.037).

The impact of **posttransplant SF** levels has also been shown to be pejorative for some outcomes after allo-HSCT. As mentioned above, shortly after transplantation, Tsat and SF levels are considerably elevated (567, 568, 611). In this early phase, the pro-oxidant properties of elevated NTBI levels can exacerbate the toxic effects of the conditioning regimen (612) as well as short- and long-term complications, including infections (fungal and bacterial) and GVHD (613). Meyer et al were the first to study the effects of SF measured at different times after allo-HSCT in 290 patients (608). Their multivariate Cox model prediction of OS, adjusted for patient age, donor type and year of transplantation, confirmed that SF levels above the median at different time points (pre-transplant, at 6, 12 and 24 months) were predictive of increased mortality in all periods analyzed. Similarly, Fingrut et al showed in 229 allo-HSCT patients that the group with high day 0 or posttransplant SF (measured between 1 month and 1 year posttransplant) had lower OS at 3 years and higher NRM (independently of pretransplant SF or GVHD), with no difference in terms of relapse (565). Multivariate analysis confirmed that posttransplant SF was an independent prognostic factor for OS (HR 2.32, p=0.001) and NRM (HR 3.91, p=0.001). However, in that study, pretransplant SF did not impact on OS or NRM. Furthermore, posttransplant but not pretransplant SF was also shown to be an independent prognostic marker for OS and NRM in patients who developed GVHD (565).

However, SF is not specific for IO and may also increase because of infection/inflammation, renal failure or hepatic cytolysis secondary to conditioning (308). Thus, while many studies claimed to show a causal relationship between IO (using ferritin as a surrogate marker) and unfavorable outcomes after allo-HSCT, these results must be interpreted with caution. Indeed, Armand et al demonstrated that LIC, more specific than SF at quantifying true IO, was very poorly predictive of posttransplant outcomes such as OS, NRM, PFS, relapse, VOD or GVHD, whereas pretransplant SF retained a negative impact on these outcomes (614). This indicates that elevated SF during allo-HSCT does not fully represent IO but probably involves other factors. Subsequently, other studies also investigated the impact of LIC (through its hepatic quantification by MRI), on morbidity and

mortality after transplantation (615-617). Indeed, these two studies demonstrated, in 45 and 88 allograft recipients, respectively, that pretransplant SF had a pejorative impact on OS and NRM whereas LIC quantified by pretransplant MRI had no significant effect on these outcomes! These results support that the prognostic impact of SF is at least partly independent of IO.

To our knowledge, no study has analyzed the impact of pretransplant serum hepcidin and ferritin on allogeneic HSCT outcomes, considering iron parameters, other biomarkers and multiple clinical characteristics (related to patient, disease, donor and transplant characteristics) in a large cohort of patients.

***Table 6. Summary of studies analyzing the impact of pretransplant serum ferritin and/or hepcidin on survival outcomes after allogeneic HSCT.*** *AL: acute leukemia; ALL: acute lymphoblastic leukemia; allo-HSCT: allogeneic hematopoietic stem cell transplantation; AML: acute myeloid leukemia; ARDS: Acute Respiratory Distress Syndrome; auto-HSCT: autologous hematopoietic stem cell transplantation; BEAM: Carmustine, Etoposide, Cytarabine, Melphalan ; BM: bone marrow; CML: chronic myeloid leukemia; CMV: cytomegalovirus; CRP: C-reactive protein; DRI: disease risk index; a/c GVHD: acute/chronic graft-versus-host disease; Hb: hemoglobin; HCT-CI: Hematopoietic Cell Transplantation-Comorbidity Index; IFI: invasive fungal infection; IO: iron overload; LDH: lactate dehydrogenase; LIC: liver iron content ; LPD: lymphoproliferative disorder; MA: myeloablative ; MDS: myelodysplastic syndrome; MM: multiple myeloma; MRI: magnetic resonance imaging; MV: multivariate analysis; N: number; NHL: non-Hodgkin lymphoma; NMA: nonmyeloablative; NRM: non-relapse mortality; NS: not significant; OS: overall survival; p-JAK2: phosphorylated Janus kinase 2; PMN: neutrophils; PFS: progression-free survival; plt: platelets; preT: pretransplant; postT: posttransplant; radioT: radiotherapy; RBC: red blood cells; RIC: reduced-intensity conditioning; sTfR: soluble transferrin receptor; SF: serum ferritin; Tsat: transferrin saturation; Tx: transfusion; Tx-depend transplant dependency. UCB: unrelated cord blood; UD: unrelated donor; UV: univariate analysis; VOD: veno-occlusive disease; y: year*

| Author, year | Characteristics of the cohort | | | | | Overall survival | Progression-free survival & relapse | Non-relapse mortality |
|---|---|---|---|---|---|---|---|---|
| | Type of study | N patients | Disease | Transplant & conditioning | Outcome measures | | | |
| ***Altes, 2002*** | Prospective | 25 | AL, CML, lymphoma | Allo & auto-HSCT MA, BEAM | OS, NRM | High SF & Tsat≥100% ➔ poor OS *(UV & MV)* | | High SF & Tsat≥100% ➔ high TRM *(UV, only Tsat in MV)* |
| ***Armand, 2007*** | Retrospective | 590 | Hematologic malignancies | Allo-HSCT MA | OS, PFS, relapse, NRM, VOD, aGVHD (5yrs) | All patients: high SF ➔ poor OS *(UV, not MV)*<br>MDS or AL: high SF ➔ poor OS *(UV & MV)*<br>CML or NHL: NS effect | All: high SF ➔ poor PFS *(UV, not MV)*<br>MDS: high SF ➔ poor PFS *(UV & MV)* but NS effect on relapse<br>Other diseases: NS effect on PFS & relapse *(UV & MV)* | All: high SF ➔ high TRM *(UV, not MV)*<br>MDS: high SF ➔ high TRM *(UV & MV)*<br>Other diseases: NS effect |
| ***Armand, 2008*** | Retrospective | 445 | AL, MDS | Allo-HSCT MA, RIC | OS | High SF (≥2,500) ➔ poor OS *(UV & MV)* | | |
| ***Kim, 2008*** | Retrospective | 38 | Hematologic malignancies | Allo-HSCT RIC | OS, PFS, NRM, VOD, infection, aGVHD, cGVHD, plt & PMN engraftment, chimerism | High SF (≥1,000) ➔ poor 3y-OS *(UV; MV not done)* | High SF & high Tsat ➔ poor 3y-PFS *(UV & MV)* | High SF ➔ NS effect |
| ***Platzbecker, 2008*** | Retrospective | 172 | MDS | Allo-HSCT MA | OS, relapse, NRM, aGVHD, cGVHD | High SF (>1,000) ➔ poor OS *(UV, not MV)*<br>Tx dependence ➔ NS effect *(UV & MV)*<br>*MV*: marrow blasts & HCT-CI ➔ poor OS | High SF ➔ NS effect on relapse | High SF ➔ NS effect |
| ***Pullarkat, 2008*** | Prospective | 190 | Hematologic malignancies | Allo-HSCT MA | OS, infection, aGVHD (day 100 & long-term) | High SF (≥1,000) ➔ poor OS *(UV & MV)*<br>*MV*: male, AML/MDS, MUD, MA ➔ poor OS<br>Diagnosis & disease status ➔ NS effect | | |
| ***Kanda, 2009*** | Retrospective | 55 | Hematologic malignancies | Allo-HSCT MA, RIC | OS, early infections, engraftment | High **hepcidin** (>50 ng/mL) ➔ NS effect | | |
| ***Kataoka, 2009*** | Retrospective | 264 | Hematologic malignancies | Allo-HSCT MA, NMA | OS, relapse, NRM, infection, aGVHD, cGVHD (5yrs) | High SF (>599) ➔ poor OS *(UV & MV)*<br>*MV*: high DRI, alemtuzumab, high-risk HCT-CI ➔ poor OS | High SF ➔ NS effect | High SF ➔ high NRM *(UV & MV)*<br>*MV*: interm. DRI, alemtuzumab, high-risk HCT-CI ➔ high NRM |
| ***Lee, 2009*** | Retrospective | 101 | Malignant & nonmalignant hematologic disorders | Allo-HSCT MA | OS, NRM, VOD, hepatic toxicity, ARDS, infections, CMV reactivation, GVHD | High SF (>1,000) ➔ poor OS *(UV & MV)* | | High SF ➔ high NRM *(UV & MV)* |
| ***Mahindra, 2009a*** | Retrospective | 64 | Hematologic malignancies | Allo-HSCT NMA | OS, relapse, NRM, GVHD | High SF (>1,615) ➔ poor OS *(UV & MV)*<br>*MV*: age, prior transplant ➔ poor OS | High SF ➔ NS effect | High SF ➔ NS effect |
| ***Mahindra, 2009b*** | Retrospective | 222 | AL, CML, MDS, NHL | Allo-HSCT MA | OS, PFS, relapse, NRM, VOD, aGVHD, cGVHD | High SF (>1,910) ➔ poor OS *(UV & MV)*<br>*MV*: N treatment lines, prior radioT, preT LDH ➔ poor OS | High SF ➔ poor PFS *(UV & MV)* but NS effect on relapse<br>*MV*: N treatment lines, prior radioT, preT bilirubin ➔ poor PFS | High SF ➔ high NRM *(UV; MV not done)* |

| Author, year | Characteristics of the cohort | | | | | Overall survival | Progression-free survival & relapse | Non-relapse mortality |
|---|---|---|---|---|---|---|---|---|
| | Type of study | N patients | Disease | Transplant & conditioning | Outcome measures | | | |
| ***Maradei, 2009*** | Retrospective | 427 | Hematologic malignancies | Auto & allo-HSCT MA, NMA | OS (5 yrs), VOD | High SF (>1,000) ➔ poor OS *(UV; MV not done)* | | |
| ***Alessandrino 2010*** | Retrospective | 129 (357) | MDS | Allo-HSCT MA, RIC | OS, relapse, NRM | High SF (4 groups) ➔ poor OS *(UV & MV)*<br>Tx-depend. & burden ➔ poor OS *(UV & MV)*<br>*MV*: age, WHO, disease status, HCT-CI, donor type ➔ poor OS | High SF ➔ NS effect<br>Tx-depend. & burden ➔ NS effect | High SF ➔ high NRM *(UV & MV)*<br>Tx-depend. & burden ➔ high NRM *(UV & MV)*<br>*MV*: age, year HSCT, HCT-CI, donor type, conditioning ➔ high NRM |
| ***Lim, 2010*** | Retrospective | 99 | MDS | Allo-HSCT RIC | OS, PFS, relapse, NRM, infections, aGvHD, cGVHD | High SF (>1,500) ➔poor 3y-OS *(UV & MV)*<br>*MV*: >5% BM blasts at transplant ➔ poor 3y-OS | High SF ➔ poor PFS *(UV, not MV)* | High SF ➔ NS effect |
| ***Oakes, 2010*** | Retrospective | 47 | Hematologic malignancies | Allo-HSCT RIC | OS, PFS, NRM | High SF (>1,000) ➔ poor 2y-OS *(UV & MV)*<br>*MV*: no other factor ➔ poor 2y-OS | High SF ➔ NS effect | High SF ➔ high 1y-NRM *(UV; MV not done)* |
| ***Sucak, 2010*** | Retrospective | 250 | Malignant & nonmalignant hematologic disorders | Allo & auto-HSCT MA, RIC | OS, NRM, toxicities, infections, aGVHD | High SF (>500) ➔ poor OS *(UV & MV)*<br>*MV*: HCT-CI ➔ poor OS | | High SF ➔ NS effect |
| ***Aki, 2011*** | Retrospective | 100 | Hematologic malignancies | Allo-HSCT MA, NMA | OS, early toxicities, infections, engraftment, transfusions | High **SF** or **hepcidin** ➔ NS effect | | |
| ***Eisfeld, 2011*** | Prospective | 42 | AML | Allo-HSCT MA, RIC | OS, PFS, relapse, NRM, RBC Tx | High **SF** or **hepcidin**, **postT SF** or **hepcidin** (3-5 mo postT) ➔ NS effect | High **SF** or **hepcidin**, **postT SF** or **hepcidin** (3-5 mo postT) ➔ NS effect | High **SF** or **hepcidin**, **postT SF** or **hepcidin** (3-5 mo postT) ➔ NS effect |
| ***Kanda, 2011*** | Retrospective | 112 | Hematologic malignancies | Allo-HSCT MA, RIC | OS, NRM, bacterial infections | High SF (>700) ➔ poor OS *(UV & MV)*<br>*MV*: recent chemo ➔ poor OS | | High SF (>700) ➔ high NRM *(UV & MV)*<br>*MV*: CRP ➔ high NRM |
| ***Tachibana, 2011a*** | Retrospective | 143 | AL | Allo-HSCT MA, RIC | OS, relapse, NRM (5 yrs) | All patients & AML: high SF (>1,000) ➔ poor OS *(UV & MV)*<br>*MV all patients*: adv. disease, UD ➔ poor OS<br>*MV AML:* adv. disease, ➔ poor OS<br>ALL: high SF ➔ NS effect | AML: high SF ➔ high relapse *(UV, MV not done)*<br>ALL: high SF ➔ NS effect | AML: high SF ➔ high NRM *(UV, MV not done)*<br>ALL: high SF ➔ NS effect |
| ***Tachibana, 2011b*** | Retrospective | 114 | AML, MDS | Allo-HSCT MA, RIC | OS, bacteremia (day 100) | High SF ➔ NS effect | | |
| ***Tachibana, 2011c*** | Retrospective | 119 | AML, MDS | Allo-HSCT MA, RIC | OS, relapse, NRM | High SF (>1000) ➔ poor OS *(UV & MV)*<br>*MV*: advanced disease ➔ poor OS | High SF ➔ poor PFS, ↑ relapse *(UV & MV)*<br>*MV*: adv. disease ➔ poor PFS, ↑ relapse | High SF ➔ NS effect |
| ***Wahlin, 2011*** | Retrospective | 309 | Hematologic malignancies | Allo-HSCT MA, RIC | OS, PFS, relapse, NRM, cGVHD | High SF (>400) ➔ poor OS *(UV & MV)*<br>*MV*: advanced disease ➔ poor OS | High SF ➔ poor PFS, ↑ relapse *(UV & MV)*<br>*MV*: advanced disease ➔ poor PFS<br>Previous transplant ➔ ↑ relapse | High SF (>400) ➔ high NRM *(UV, not MV)*<br>*MV*: advanced disease ➔ high NRM |
| ***Aki, 2012*** | Retrospective | 106 | AL | Allo-HSCT MA | OS, PFS, NRM, toxicities | High SF (>1,500) ➔ poor OS *(UV, not MV)* | High SF ➔ NS effect | High SF ➔ NS effect |
| ***Armand, 2012*** | Prospective | 45 | AL, MDS | Allo-HSCT MA | OS, PFS, relapse, NRM, VOD, GVHD | High SF (>2,500, not >1000) ➔ poor OS *(UV & MV)*<br>High MRI LIC ➔ NS effect | High SF ➔ NS effect<br>High MRI LIC ➔ NS effect | High SF ➔ NS effect<br>High MRI LIC ➔ NS effect |

| Author, year | Characteristics of the cohort | | | | | Overall survival | Progression-free survival & relapse | Non-relapse mortality |
|---|---|---|---|---|---|---|---|---|
| | Type of study | N patients | Disease | Transplant & conditioning | Outcome measures | | | |
| ***Bazuaye, 2012*** | Retrospective | 230 | Malignant & nonmalignant hematologic disorders | Allo-HSCT MA | OS (5 yrs) | High SF ➔ poor OS *(UV & MV)*<br>*MV*: high Tsat, low sTfR, low Tf ➔ poor OS | | |
| ***Sivgin, 2012*** | Retrospective | 84 | Malignant & nonmalignant hematologic disorders | Allo-HSCT MA, NMA | OS, PFS, relapse, infections, aGVHD, cGVHD, plt & PMN engraftment | High SF (>1,000) ➔ poor OS *(UV & MV)*<br>*MV*: Tx-depend. >12 mo, >1 MM ➔ poor OS | High SF ➔ poor PFS *(UV & MV)*<br>*MV*: Tx-depend. >12 mo, >1 MM ➔poor PFS | |
| ***Tachibana, 2012*** | Retrospective | 261 | AL, MDS | Allo-HSCT MA, RIC | OS, PFS, NRM (5 yrs) | High SF (>1,000) ➔ poor OS *(UV & MV)*<br>*MV*: advanced disease, UCB ➔ poor OS | High SF ➔ poor PFS *(UV & MV)*<br>*MV*: adv. disease, UCB ➔ poor PFS | High SF ➔ high NRM *(UV & MV)*<br>*MV*: adv. disease, UCB, UD ➔ high NRM |
| ***Tanaka, 2012*** | Retrospective | 47 | AML, MDS | Allo-HSCT MA, RIC | OS, PFS, relapse, NRM | High SF (>1000) ➔ poor OS *(UV & MV)*<br>*MV*: advanced disease ➔ poor OS | High SF ➔ poor PFS, ↑ relapse *(UV & MV)*<br>*MV*: adv. disease ➔ poor PFS, ↑ relapse | High SF ➔ NS effect |
| ***Wermke. 2012*** | Prospective | 88 | AML, MDS | Allo-HSCT MA, RIC | OS, relapse, NRM, liver & cardiac toxicity, infections, IFI, aGVHD | High SF (≥ 2500) ➔ NS effect<br>High MRI LIC (≥ 125 µmol/gr) ➔ poor OS *(UV & MV)*<br>*MV*: age, time from diagnosis, DRI, HCT-CI, MA, UD ➔ poor OS | High SF ➔ NS effect<br>High MRI LIC ➔ NS effect | High SF ➔ NS effect<br>High MRI LIC ➔ high NRM *(UV & MV)*<br>*MV*: age, time from diagnosis, DRI, HCT-CI, MA, UD ➔ high NRM |
| ***Barba, 2013*** | Retrospective | 201 | LPD, MM | Allo-HSCT RIC | OS, relapse, NRM, infectious mortality, engraftment | High SF (>399) ➔ poor OS *(UV & MV)*<br>*MV*: age>60, female, HCT-CI>2 ➔ poor OS | High SF ➔ NS effect | High SF ➔ high NRM *(UV & MV)*<br>*MV*: age>60, female, MM or NHL, HCT-CI>2 ➔ high NRM |
| ***Meyer, 2013*** | Retrospective | 290 | Malignant & nonmalignant hematologic disorders | Allo-HSCT MA, RIC | OS, relapse, NRM, GHVH | High SF (>1,332) ➔ poor OS *(UV & MV)*<br>High **postT** SF (0-6-12-24 mo) ➔ poor OS *(UV, MV not done)*<br>*UV*: high Tsat or SeFe, low sTfR ➔ poor OS | High SF ➔ high relapse *(UV, MV not done)*<br>High **postT** SF ➔ high relapse *(UV, MV not done)* | High SF ➔ high NRM *(UV, MV not done)*<br>High **postT** SF ➔ high NRM *(UV, MV not done)* |
| ***Sakamoto, 2013*** | Retrospective | 211 | Malignant & nonmalignant hematologic disorders | Allo-HSCT MA, RIC | OS, NRM, aGVHD, cGVHD | High SF (>880) ➔ poor OS *(UV & MV)*<br>*MV*: CRP, age>50, female, high DRI ➔ poor OS | | High SF ➔ high NRM *(UV & MV)*<br>*MV*: CRP, female ➔ high NRM |
| ***Tachibana, 2013*** | Retrospective | 153 | AML, MDS | Allo-HSCT MA, RIC | OS, PFS, relapse, NRM | High SF (>1000) ➔ poor OS *(UV & MV)*<br>*MV*: advanced disease ➔ poor OS | Score (high SF & advanced disease) ➔ poor PFS, ↑ relapse | Score (high SF & advanced disease) ➔ NS effect |
| ***Trottier, 2013*** | Prospective | 88 | Malignant & nonmalignant hematologic disorders | Allo-HSCT MA, RIC | OS, relapse, NRM, VOD, organ failure, infections, GVHD (1 yr) | High SF (>1,000) ➔ poor 1y-OS *(UV & MV)*<br>High MRI LIC (>5 mg/gr) ➔ NS effect | High MRI LIC ➔ NS effect | High SF ➔ high NRM *(UV & MV)*<br>High MRI LIC ➔ NS effect |
| ***Virtanen, 2013*** | Prospective | 68 | Malignant & nonmalignant hematologic disorders | Allo-HSCT MA, RIC | OS, NRM, infections, aGVHD, cGVHD | High MRI LIC (>150 µmol/gr) ➔ NS effect | | High MRI LIC ➔ NS effect |
| ***Boehm, 2014*** | Retrospective | 66 | MDS, secondary AML | Allo-HSCT MA, RIC | OS, PFS, relapse, NRM | High SF (>1,000) ➔ poor OS *(UV & MV)* | High SF ➔ poor PFS *(UV & MV)*<br>High SF ➔ high relapse *(UV & MV)* | High SF ➔ NS effect |
| ***Konuma, 2014*** | Retrospective | 133 | AL, MDS | Allo-HSCT MA | OS, relapse, aGVHD, cGVHD, PMN engraftment | High SF ➔ NS effect | High SF ➔ NS effect | |
| ***Matsumoto, 2014*** | Retrospective | 54 | AL | Allo-HSCT MA, RIC | OS | High SF (>1,000) ➔ poor OS *(UV & MV)* | | |

| Author, year | Characteristics of the cohort | | | | | Overall survival | Progression-free survival & relapse | Non-relapse mortality |
|---|---|---|---|---|---|---|---|---|
| | Type of study | N patients | Disease | Transplant & conditioning | Outcome measures | | | |
| ***Oran, 2014*** | Retrospective | 256 | MDS | Allo-HSCT MA, RIC | OS, PFS, relapse, NRM | High SF (>1,130) ➔ poor OS *(UV & MV)* | High SF ➔ poor PFS *(UV & MV)*<br>High SF ➔ NS effect on relapse | High SF ➔ high NRM *(UV, not MV)* |
| ***Tachibana, 2014*** | Retrospective | 110 | Hematologic malignancies | Allo-HSCT MA, RIC | OS, NRM | High SF (>1,000) ➔ NS effect<br>High **1y-postT SF** ➔ poor OS *(UV & MV)* | | High SF ➔ NS effect<br>High **1y-postT SF**➔poor NRM *(UV & MV)* |
| ***Jang, 2015*** | Retrospective | 74 | AML | Allo-HSCT MA, RIC | OS, PFS, relapse | High SF (>1,400) ➔ NS effect | High SF (>1,400) ➔ high relapse<br>High SF (>1,400) ➔ NS effect on PFS | |
| ***Tanaka, 2015*** | Retrospective | 190 | AML, MDS | Allo-HSCT MA, RIC, NMA | OS, relapse, NRM, infections, aGVHD, cGVHD, PMN engraftment | High SF (>1,000) ➔ poor OS *(UV & MV)*<br>*MV*: high DRI ➔ poor OS | High SF ➔ NS effect | High SF ➔ NS effect |
| ***Vaughn, 2015*** | Retrospective | 3917 | Malignant & nonmalignant hematologic disorders | Allo-HSCT MA, RIC, NMA | OS, NRM | High SF (>2,500) ➔ poor OS *(UV & MV)*<br>*MV*: high SF, low albumin, low plt and high HCT-CI ➔ poor OS *(UV & MV)* | | High SF (>2,500) ➔ high NRM *(UV & MV)*<br>*MV*: high SF, low albumin, low plt and high HCT-CI ➔ high NRM *(UV & MV)* |
| ***Artz, 2016*** | Retrospective | 784 | AML, MDS | Allo-HSCT MA | OS, relapse, NRM, aGVHD, cGVHD | High SF (>2,500) ➔ NS effect<br>*MV*: high SF included in 3-biomarker score with high CRP & low albumin ➔ poor OS | High SF ➔ NS effect | High SF ➔ NS effect<br>*MV*: high SF included in 3-biomarker score with high CRP & low albumin ➔ high NRM |
| ***Cremers, 2016*** | Retrospective | 201 | AL, lymphoma | Allo-HSCT MA, NMA | OS, relapse, NRM | High SF (>1,000) ➔ NS effect<br>High Tx burden ➔ poor OS *(UV & MV)* | High SF ➔ NS effect<br>High Tx burden ➔ high relapse *(UV & MV)* | High SF ➔ NS effect<br>High Tx burden ➔ high NRM *(UV & MV)* |
| ***Sivgin, 2016*** | Retrospective | 125 | Hematologic malignancies | Allo-HSCT MA, RIC | OS, PFS | High SF (>1,400) ➔ NS effect | High SF ➔ NS effect | |
| ***Chee, 2017*** | Retrospective | 602 | Hematologic malignancies | Allo-HSCT MA, NMA, RIC | OS, relapse, NRM | High SF (>1000) ➔ poor OS *(UV & MV)*<br>*MV*: low albumin or Hb, high DRI ➔ poor OS | High SF ➔ high 1y-relapse *(UV & MV)*<br>*MV*: high DRI ➔ high 1y- relapse | High SF ➔ high 1-yr NRM *(UV & MV)*<br>*MV*: age>60, low Hb ➔ high NRM |
| ***Sakamoto, 2017*** | Retrospective | 166 | AML, MDS | Allo-HSCT MA, RIC, NMA | OS, PFS, NRM, toxicities, aGVHD, engraftment | High **SF** (>694) & **hepcidin** (>35 ng/mL) ➔ poor 3y-OS *(UV)*<br>*MV*: high hepcidin, disease status, male ➔ poor 3y-OS (SF not tested) | High **SF** & **hepcidin** ➔ NS effect | High **SF** & **hepcidin** ➔ NS effect |
| ***Fingrut, 2021*** | Retrospective | 229 | Malignant & nonmalignant hematologic disorders | Allo-HSCT MA, RIC | OS, relapse, NRM, GVHD | High SF ➔ NS effect<br>High **postT** SF (1 mo-1 yr post & on day 0) ➔ poor 3y-OS *(UV & MV)* | High SF ➔ NS effect<br>High **postT** SF ➔ NS effect | High SF ➔ NS effect<br>High **postT** SF ➔ high NRM *(UV & MV)*<br>*MV:* aGVHD ➔ high NRM |
| ***Dudda, 2022*** | Retrospective | 102 | MDS | Allo-HSCT MA, RIC | OS, PFS | High SF (>1,000) ➔ poor OS *(UV & MV)* | High SF ➔ poor PFS *(UV & MV)* | |

***Table 7. Summary of studies analyzing the impact of pretransplant serum ferritin and/or hepcidin on toxicities after allogeneic HSCT.*** *AML: acute myeloid leukemia; allo-HSCT: allogeneic hematopoietic stem cell transplantation; ARDS: acute respiratory distress syndrome; auto-HSCT: autologous hematopoietic stem cell transplantation; BEAM: Carmustine, Etoposide, Cytarabine, Melphalan; BM: bone marrow; bacteriemia; CMV reactivation/disease; CML: chronic myeloid leukemia; CR: complete remission; CRP: C-reactive protein; a/c GVHD: acute/chronic graft-versus-host disease; DLCO: diffusing capacity of the lungs for carbon monoxide; HCT-CI: Hematopoietic Cell Transplantation-Comorbidity Index; IFI: invasive fungal infection; IPS: idiopathic pneumonia syndrome ; LIC: liver iron content; MA: myeloablative; MM: multiple myeloma; MRI: magnetic resonance imaging; MV: multivariate analysis; N: number; NS: not significant; NRM: non-relapse mortality; OS: overall survival; plt: platelets; PMN engraftment; postT: posttransplant; preT: pretransplant; PFS: progression-free survival; RBC Tx; RIC: reduced-intensity conditioning; SF: serum ferritin; TBI: total body irradiation; tox: toxicity; Tx: transfusion; UD: unrelated donor; UV: univariate analysis; VOD: veno-occlusive disease; y: year*

| Author, year | Characteristics of the cohort | | | | | Toxicities | Infections | Acute / chronic GVHD |
|---|---|---|---|---|---|---|---|---|
| | Type of study | N patients | Disease | Transplant & conditioning | Outcome measures | | | |
| ***Altes, 2007*** | Prospective | 81 | AL, MDS, lymphoma, MM | Allo & auto-HSCT MA, RIC, BEAM | Mucositis, bacteriemia, N febrile days | High SF (>1,500) ➔ ↑ mucositis *(UV & MV)*<br>Allo: high SF ➔ NS effect<br>Auto: high SF ➔ ↑ mucositis *(UV & MV)* | High SF ➔↑ bacteremia & fever days *(UV & MV)*<br>*MV*: TBI ➔ ↑ bacteriemia<br>*MV*: auto, neutropenia duration ➔ ↑ fever days<br>Allo: high SF ➔ NS effect<br>Auto: high SF ➔ ↑ bacteremia & fever days *(UV & MV)* | |
| ***Armand, 2007*** | Retrospective | 590 | Hematologic malignancies | Allo-HSCT MA | OS, PFS, relapse, NRM, VOD, aGVHD (5 yrs) | High SF ➔ NS effect on VOD | | High SF ➔ NS effect on aGVHD |
| ***Kim, 2008*** | Retrospective | 38 | Hematologic malignancies | Allo-HSCT RIC | OS, PFS, NRM, VOD, infection, aGVHD, cGVHD, plt & PMN engraftment, chimerism | High SF (≥1,000 ng/mL) ➔ NS effect on VOD | High SF ➔ NS effect on infection, CMV reactivation/disease | High SF ➔ NS effect on a/cGVHD |
| ***Platzbecker, 2008*** | Retrospective | 172 | MDS | Allo-HSCT MA | OS, relapse, NRM, aGVHD, cGVHD | | | High SF (≥2,500 ng/mL) ➔ ↑ grade 3-4 aGVHD *(UV, MV not done)* ➔ NS effect on cGVHD |
| ***Pullarkat, 2008*** | Prospective | 190 | Hematologic malignancies | Allo-HSCT MA | OS, infection, aGVHD (day 100 & long-term) | | High SF (>1,000mg/mL) ➔ ↑ bacteremia *(UV & MV)* | High SF ➔ NS effect on aGVHD |
| ***Kanda, 2009*** | Retrospective | 55 | Hematologic malignancies | Allo-HSCT MA, RIC | OS, early infections, engraftment | | High **hepcidin** (>50 ng/mL) ➔ ↑ bacterial infections *(UV & MV)* ➔ NS effect on CMV reactivation | |
| ***Kataoka, 2009*** | Retrospective | 264 | Hematologic malignancies | Allo-HSCT MA, NMA | OS, NRM, relapse, infections, aGVHD, cGVHD (5 yrs) | | High SF (≥599 ng/mL) ➔ ↑ infections *(UV, MV not done)* | High SF ➔ NS effect on a/cGVHD |
| ***Lee, 2009*** | Retrospective | 101 | Malignant & nonmalignant hematologic disorders | Allo-HSCT MA | OS, NRM, VOD, hepatic toxicity, ARDS, infections, CMV reactivation, aGVHD | High SF (>1,000 ng/mL) ➔ ↑ ARDS *(UV, not MV)* ➔ NS effect on VOD or hepatic tox | High SF ➔ NS effect on bacteriemia or CMV reactivation | High SF ➔ NS effect on aGVHD |
| ***Mahindra, 2009 (a)*** | Retrospective | 64 | Hematologic malignancies | Allo-HSCT NMA | OS, relapse, NRM, GVHD | | | High SF (>1,615 ng/mL) ➔ NS effect on a/cGVHD |
| ***Mahindra, 2009 (b)*** | Retrospective | 222 | AL, CML, MDS, NHL | Allo-HSCT MA | OS, PFS, relapse, NRM, VOD, aGVHD, cGVHD | High SF (>1,910 ng/mL) ➔ NS effect on VOD | | High SF ➔ ↓ aGVHD & cGVHD *(UV, MV not done)* |

| Author, year | Characteristics of the cohort | | | | | Toxicities | Infections | Acute / chronic GVHD |
|---|---|---|---|---|---|---|---|---|
| | Type of study | N patients | Disease | Transplant & conditioning | Outcome measures | | | |
| ***Maradei, 2009*** | Retrospective | 427 | Hematologic malignancies | Auto & allo-HSCT MA, NMA | OS (5 yrs), VOD | High SF (>1,000 ng/mL) ➔ ↑ VOD *(UV & MV)*<br>*MV*: allo, imatinib, busulfan ➔ ↑ VOD | | |
| ***Lim, 2010*** | Retrospective | 99 | MDS | Allo-HSCT RIC | OS, PFS, relapse, NRM, infections, aGvHD, cGVHD | | High SF (>1,500 ng/mL) ➔ NS effect | High SF ➔ NS effect on a/cGVHD |
| ***Ozyilmaz, 2010*** | Retrospective | 148 | Malignant & nonmalignant hematologic disorders | Allo-HSCT MA | VOD, fungal pneumonia | High SF (>1,000 ng/mL) ➔ ↑ VOD *(UV, MV not done)* | High SF ➔ ↑ fungal pneumonia *(UV & MV)*<br>*MV*: VOD ➔ ↑ fungal pneumonia | |
| ***Sucak, 2010*** | Retrospective | 250 | Malignant & nonmalignant hematologic disorders | Allo & auto-HSCT MA, RIC | OS, NRM, toxicities, infections, aGVHD | High SF (>500) ➔ ↑ mucositis *(UV, MV not done)*<br>Allo: high SF ➔ ↑ VOD *(UV, MV not done)*, NS effect on mucositis | Allo: high SF<br>➔ ↑ IFI *(UV, MV not done)*<br>➔ NS effect on pneumonia / bacteriemia | High SF ➔ NS effect on aGVHD |
| ***Aki, 2011*** | Retrospective | 100 | Hematologic malignancies | Allo-HSCT MA, NMA | OS, early toxicities, infections, engraftment, transfusions | High **SF**<br>➔↑ VOD & liver tox *(UV, MV not done)*<br>➔ NS effect on other toxicities<br>High **hepcidin**<br>➔ ↑ mucositis *(UV, MV not done)*<br>➔ NS effect on VOD & other toxicities | High **SF** & high **hepcidin**<br>➔ ↑ neutropenic fever & N febrile days<br>*(UV, MV not done)* | |
| ***Eisfeld, 2011*** | Prospective | 42 | AML | Allo-HSCT MA, RIC | OS, PFS, relapse, NRM, RBC Tx | | High **SF** or **hepcidin**, **postT SF** or **hepcidin** (3-5 mo postT) ➔ NS effect | High **SF** or **hepcidin**, **postT SF** or **hepcidin** (3-5 mo postT) ➔ NS effect |
| ***Kanda, 2011*** | Retrospective | 112 | Hematologic malignancies | Allo-HSCT MA, RIC | OS, NRM, bacterial infections | | High SF (>700 ng/mL) ➔ ↑ bacterial infections *(UV & MV)*<br>*MV*: high preT CRP ➔ ↑ bacterial infections | |
| ***Tachibana, 2011b*** | Retrospective | 114 | AML, MDS | Allo-HSCT MA, RIC | OS, bacteremia (day 100) | | High SF ➔ ↑ bacteremia *(UV & MV)* | |
| ***Wahlin, 2011*** | Retrospective | 309 | Hematologic malignancies | Allo-HSCT MA, RIC | OS, PFS, relapse, NRM, cGVHD | | | High SF (>400 ng/mL) ➔ ↑ cGVHD *(UV & MV)*<br>*MV*: previous transplant, UD, transplant centre ➔ ↑ cGVHD |
| ***Aki, 2012*** | Retrospective | 106 | AL | Allo-HSCT MA | OS, PFS, NRM, toxicities | High SF (>1,500 ng/mL) ➔ NS effect on toxicities | | |
| ***Armand, 2012*** | Prospective | 45 | AL, MDS | Allo-HSCT MA | OS, PFS, relapse, NRM, VOD, GVHD | High MRI LIC ➔ NS effect on VOD | | High MRI LIC ➔ NS effect on a/cGVHD |
| ***Sivgin, 2012*** | Retrospective | 84 | Malignant & nonmalignant hematologic disorders | Allo-HSCT MA, NMA | OS, PFS, relapse, infections, aGVHD, cGVHD, plt & PMN engraftment | | High SF (>1,000 ng/mL) ➔ ↑ infections *(UV, MV not done)* | High SF ➔ NS effect on a/cGVHD |

| Author, year | Characteristics of the cohort | | | | | Toxicities | Infections | Acute / chronic GVHD |
|---|---|---|---|---|---|---|---|---|
| | Type of study | N patients | Disease | Transplant & conditioning | Outcome measures | | | |
| ***Wermke, 2012*** | Retrospective | 88 | AML, MDS | Allo-HSCT MA, RIC | OS, relapse, NRM, liver & cardiac toxicity, infections, IFI, aGVHD | High SF (≥ 2500 ng/ml) ➔ NS effect on toxicities<br>High MRI LIC (≥ 125 µmol/gr) ➔ NS effect on toxicities | High SF ➔ NS effect on bacterial infect. or IFI<br>High MRI LIC ➔ NS effect on bacterial infections or IFI | High SF ➔ NS effect on aGVHD<br>High MRI LIC ➔ NS effect on aGVHD |
| ***Barba, 2013*** | Retrospective | 201 | LPD, MM | Allo-HSCT MA, RIC | OS, relapse, NRM, infectious mortality, engraftment | | High SF (>399 ng/mL) ➔ ↑ infectious mortality *(UV & MV)*<br>*MV*: age>60, female ➔ ↑ infectious mortality | |
| ***Goto, 2013*** | Retrospective | 122 | Malignant & nonmalignant hematologic disorders | Allo-HSCT MA | Hepatic toxicity, diabetes | High SF (>1,000 ng/mL) ➔ ↑ hepatic tox & diabetes *(UV & MV)*<br>*MV*: HLA mismatch ➔ ↑ hepatic tox<br>*MV*: age, steroids, male ➔ ↑ diabetes | | |
| ***Meyer, 2013*** | Retrospective | 290 | Malignant & nonmalignant hematologic disorders | Allo-HSCT MA, RIC | OS, relapse, NRM, GHVH | | | High SF (>1,332ng/mL) ➔ ↑ aGVHD & cGVHD *(UV, MV not done)*<br>High **postT** SF (0-6-12-24 mo) ➔ ↑ aGVHD & cGVHD *(UV, MV not done)* |
| ***Nishimoto, 2013*** | Retrospective | 63 | Malignant & nonmalignant hematologic disorders | Allo-HSCT MA, RIC | Cardiac toxicity | High SF ➔ ↑ cardiac tox *(UV & MV)*<br>*MV*: MA ➔ ↑ cardiac tox | | |
| ***Sakamoto, 2013*** | Retrospective | 211 | Malignant & nonmalignant hematologic disorders | Allo-HSCT MA, RIC | OS, NRM, aGVHD, cGVHD | | | High SF ➔ NS effect on a/cGVHD<br>AML: high SF ➔ ↓ cGVHD<br>*(UV, MV not done)* |
| ***Sivgin, 2013*** | Retrospective | 73 | Malignant & nonmalignant hematologic disorders | Allo-HSCT MA, NMA | Fungal pneumonia | | High SF ➔ ↑ fungal pneumonia<br>*(UV, MV not done)* | |
| ***Trottier, 2013*** | Retrospective | 88 | Malignant & nonmalignant hematologic disorders | Allo-HSCT MA, RIC | OS, relapse, NRM, VOD, organ failure, infections, GVHD (1 yr) | High SF (>1,000ng/mL) ➔ NS effect on VOD or organ failure<br>High MRI LIC (>5 mg/gr) ➔ NS effect on VOD or organ failure | High SF ➔ NS effect on infections<br>High MRI LIC ➔ NS effect on infections | High SF ➔ NS effect on a/cGVHD<br>High MRI LIC ➔ NS effect on a/cGVHD |
| ***Virtanen, 2013*** | Prospective | 68 | Malignant & nonmalignant hematologic disorders | Allo-HSCT MA, RIC | OS, NRM, infections, aGVHD, cGVHD | | High MRI LIC (>150 µmol/gr) ➔ ↑ infections *(UV, MV not done)* | High MRI LIC ➔ ↓ aGVHD *(UV, MV not done)* & ↓ cGVHD *(UV & MV)* |
| ***Konuma, 2014*** | Retrospective | 133 | AL, MDS | Allo-HSCT MA | OS, relapse, aGVHD, cGVHD, PMN engraftment | | | High SF ➔ NS effect on a/cGVHD |
| ***Dadwal, 2015*** | Retrospective | 477 | Hematologic malignancies | Allo-HSCT MA | IFI | | High SF ➔ ↑ IFI (UV & MV) in all patients & RIC (NS effect in MA)<br>MV: aGVHD ➔ ↑ IFI | |
| ***Tanaka, 2015*** | Retrospective | 190 | AML, MDS | Allo-HSCT MA, RIC, NMA | OS, relapse, NRM, bloodstream / bacterial / fungal infections, aGVHD, cGVHD, PMN engraftment | | High SF (>1,000 ng/mL) ➔ NS effect on bloodstream / bacterial / fungal infections | High SF ➔ NS effect on aGVHD ➔ ↑ cGVHD *(UV & MV)* |

| Author, year | Characteristics of the cohort | | | | | Toxicities | Infections | Acute / chronic GVHD |
|---|---|---|---|---|---|---|---|---|
| | Type of study | N patients | Disease | Transplant & conditioning | Outcome measures | | | |
| ***Artz, 2016*** | Retrospective | 784 | AML, MDS | Allo-HSCT MA | OS, relapse, NRM, aGVHD, cGVHD | | | High SF ➔ NS effect on aGVHD or cGVHD |
| ***Sakamoto, 2017*** | Retrospective | 166 | AML, MDS | Allo-HSCT MA, RIC, NMA | OS, PFS, NRM, toxicities, aGVHD, engraftment | | | High **SF** (>694 mg/mL) & **hepcidin** (>35 ng/mL) ➔ NS effect on aGVHD |
| ***Fingrut, 2021*** | Retrospective | 229 | Malignant & nonmalignant hematologic disorders | Allo-HSCT MA, RIC | OS, relapse, NRM, GVHD | | | High SF ➔ NS effect on a/c GVHD<br>High **postT** SF ➔ ↑ aGVHD *(UV, MV not done)*, NS effect on cGVHD |
| ***Tamaki, 2022*** | Retrospective | 444 | Malignant & nonmalignant hematologic disorders | Allo-HSCT MA, RIC | Idiopathic pneumonia syndrome | High SF ➔ ↑ IPS & clinical IPS *(UV & MV)*<br>*MV*: age, MA conditioning, low DLCO, 2nd allo for graft failure ➔ ↑ clinical IPS | | |

***Table 8. Summary of studies analyzing impact of pretransplant serum ferritin and/or hepcidin on posttransplant transfusions and on neutrophil, platelet and Hb engraftment after allogeneic HSCT**. AML: acute myeloid; AL: acute leukemia; allo-HSCT: allogeneic hematopoietic stem cell transplantation; ARDS: acute respiratory distress syndrome; auto-HSCT: autologous hematopoietic stem cell transplantation; BEAM: Carmustine, Etoposide, Cytarabine, Melphalan; BM: bone marrow; chimerism; CMV reactivation/disease; CR: complete remission; CRP: C-reactive protein; DRI: disease risk index; a/c GVHD: acute/chronic graft-versus-host disease; Hb: hemoglobin; HCT-CI: Hematopoietic Cell Transplantation-Comorbidity Index; IFI: invasive fungal infection; IO: iron overload; LIC: liver iron content; MA: myeloablative; MDS: myelodysplastic syndrome; MM: multiple myeloma; MV: multivariate analysis; N: number; NMA: nonmyeloablative; NS: not significant; NRM: non-relapse mortality; OS: overall survival; plt: platelets; PMN: neutrophils; postT: posttransplant; preT: pretransplant; PFS: progression-free survival; RBC: red blood cells; RBC Tx; RIC: reduced-intensity conditioning; SF: serum ferritin; TBI: total body irradiation; Tx: transfusion; UD: unrelated donor; UV: univariate analysis; VOD: veno-occlusive disease; y: year*

| Author, year | Characteristics of the cohort | | | | | RBC and/or Plt transfusions | Engraftment |
|---|---|---|---|---|---|---|---|
| | Type of study | N patients | Disease | Transplant & conditioning | Outcome measures | | |
| ***Kim, 2008*** | Retrospective | 38 | Hematologic malignancies | Allo-HSCT RIC | OS, PFS, NRM, VOD, infection, aGVHD, cGVHD, PMN & plt engraftment, chimerism | | High SF (>1,000ng/mL) ➔ NS effect on PMN or plt engraftment |
| ***Kanda, 2009*** | Retrospective | 55 | Hematologic malignancies | Allo-HSCT MA, RIC | OS, early infections, PMN engraftment | | High **hepcidin** (>50 ng/mL) ➔ NS effect on PMN engraftment |
| ***Aki, 2011*** | Retrospective | 100 | Hematologic malignancies | Allo-HSCT MA, NMA | OS, early toxicities, infections, engraftment, transfusions | High SF ➔ ↑ RBC or plt Tx *(UV, MV not done)*<br>High **hepcidin** ➔ NS effect on RBC or plt Tx | High SF ➔ ↓ PMN *(UV, MV not done)*, NS effect on plt engraftment<br>High **hepcidin** ➔ NS effect on PMN or plt engraftment |
| ***Eisfeld, 2011*** | Prospective | 42 | AML | Allo-HSCT MA, RIC | OS, PFS, NRM, relapse, RBC Tx | High **postT SF** or **hepcidin** (3-5 mo postT) ➔ ↑ RBC Tx *(UV, MV not done)* | |
| ***Sivgin, 2012*** | Retrospective | 84 | Malignant & nonmalignant hematologic disorders | Allo-HSCT MA, NMA | OS, PFS, relapse, infections, aGVHD, cGVHD, plt & PMN engraftment | | High SF (>1,000 ng/mL) ➔ NS effect on PMN or plt engraftment |
| ***Barba, 2013*** | Retrospective | 201 | LPD, MM | Allo-HSCT RIC | OS, relapse, NRM, infectious mortality, engraftment | | High SF (>399 ng/mL) ➔ ↓ PMN *(UV, MV not done)* but NS effect on plt engraftment |
| ***Konuma, 2014*** | Retrospective | 133 | AL, MDS | Allo-HSCT MA | OS, relapse, aGVHD, cGVHD, PMN engraftment | | High SF (>1,000 ng/mL) ➔ NS effect on PMN engraftment |
| ***Tanaka, 2015*** | Retrospective | 190 | AML, MDS | Allo-HSCT MA, RIC, NMA | OS, relapse, NRM, infections, aGVHD, cGVHD, PMN engraftment | | High SF (>1,000 ng/mL) ➔ ↓ PMN engraftment *(UV & MV)* |
| ***Sakamoto, 2017*** | Retrospective | 166 | AML, MDS | Allo-HSCT MA, RIC, NMA | OS, PFS, NRM, toxicities, aGVHD, engraftment | | High **SF** (>694 mg/mL) & **hepcidin** (>35 ng/mL) ➔ NS effect on PMN or Hb engraftment ➔ ↓ plt engraftment *(UV)*<br>*MV*: high **hepcidin**, high DRI, male, UCB ➔ ↓ plt engraftment |

# REFERENCES


1. Dzierzak E, Philipsen S. Erythropoiesis: development and differentiation. Cold Spring Harb Perspect Med. 2013;3(4):a011601.
2. Hattangadi SM, Wong P, Zhang L, Flygare J, Lodish HF. From stem cell to red cell: regulation of erythropoiesis at multiple levels by multiple proteins, RNAs, and chromatin modifications. Blood. 2011;118(24):6258-68.
3. Eckardt KU, Kurtz A. Regulation of erythropoietin production. Eur J Clin Invest. 2005;35 Suppl 3:13-9.
4. Singh VK, Saini A, Kalsan M, Kumar N, Chandra R. Stage-Specific Regulation of Erythropoiesis and Its Implications in. J Stem Cells. 2016;11(3):149-69.
5. Woolthuis CM, Park CY. Hematopoietic stem/progenitor cell commitment to the megakaryocyte lineage. Blood. 2016;127(10):1242-8.
6. Lodish H, Flygare J, Chou S. From stem cell to erythroblast: regulation of red cell production at multiple levels by multiple hormones. IUBMB Life. 2010;62(7):492-6.
7. Broxmeyer HE. Erythropoietin: multiple targets, actions, and modifying influences for biological and clinical consideration. J Exp Med. 2013;210(2):205-8.
8. Koury MJ. Abnormal erythropoiesis and the pathophysiology of chronic anemia. Blood Rev. 2014;28(2):49-66.
9. Bessis M. [Erythroblastic island, functional unity of bone marrow]. Rev Hematol. 1958;13(1):8-11.
10. Chasis JA, Mohandas N. Erythroblastic islands: niches for erythropoiesis. Blood. 2008;112(3):470-8.
11. Hanspal M, Smockova Y, Uong Q. Molecular identification and functional characterization of a novel protein that mediates the attachment of erythroblasts to macrophages. Blood. 1998;92(8):2940-50.
12. Soni S, Bala S, Gwynn B, Sahr KE, Peters LL, Hanspal M. Absence of erythroblast macrophage protein (Emp) leads to failure of erythroblast nuclear extrusion. J Biol Chem. 2006;281(29):20181-9.
13. Sadahira Y, Yoshino T, Monobe Y. Very late activation antigen 4-vascular cell adhesion molecule 1 interaction is involved in the formation of erythroblastic islands. J Exp Med. 1995;181(1):411-5.
14. Lee G, Lo A, Short SA, Mankelow TJ, Spring F, Parsons SF, et al. Targeted gene deletion demonstrates that the cell adhesion molecule ICAM-4 is critical for erythroblastic island formation. Blood. 2006;108(6):2064-71.
15. Anselmo A, Lauranzano E, Soldani C, Ploia C, Angioni R, D'Amico G, et al. Identification of a novel agrin-dependent pathway in cell signaling and adhesion within the erythroid niche. Cell Death Differ. 2016;23(8):1322-30.
16. Zhang H, Wan GZ, Wang YY, Chen W, Guan JZ. The role of erythrocytes and erythroid progenitor cells in tumors. Open Life Sci. 2022;17(1):1641-56.
17. Testa U. Apoptotic mechanisms in the control of erythropoiesis. Leukemia. 2004;18(7):1176-99.
18. Bernstein ID, Andrews RG, Zsebo KM. Recombinant human stem cell factor enhances the formation of colonies by CD34+ and CD34+lin- cells, and the generation of colony-forming cell progeny from CD34+lin- cells cultured with interleukin-3, granulocyte colony-stimulating factor, or granulocyte-macrophage colony-stimulating factor. Blood. 1991;77(11):2316-21.
19. Bunn HF. Erythropoietin. Cold Spring Harb Perspect Med. 2013;3(3):a011619.
20. Simon MC, Pevny L, Wiles MV, Keller G, Costantini F, Orkin SH. Rescue of erythroid development in gene targeted GATA-1- mouse embryonic stem cells. Nat Genet. 1992;1(2):92-8.
21. Gutiérrez L, Caballero N, Fernández-Calleja L, Karkoulia E, Strouboulis J. Regulation of GATA1 levels in erythropoiesis. IUBMB Life. 2020;72(1):89-105.

22. Bresnick EH, Hewitt KJ, Mehta C, Keles S, Paulson RF, Johnson KD. Mechanisms of erythrocyte development and regeneration: implications for regenerative medicine and beyond. Development. 2018;145(1).
23. Valent P, Büsche G, Theurl I, Uras IZ, Germing U, Stauder R, et al. Normal and pathological erythropoiesis in adults: from gene regulation to targeted treatment concepts. Haematologica. 2018;103(10):1593-603.
24. Gregory T, Yu C, Ma A, Orkin SH, Blobel GA, Weiss MJ. GATA-1 and erythropoietin cooperate to promote erythroid cell survival by regulating bcl-xL expression. Blood. 1999;94(1):87-96.
25. Ribeil JA, Zermati Y, Vandekerckhove J, Cathelin S, Kersual J, Dussiot M, et al. Hsp70 regulates erythropoiesis by preventing caspase-3-mediated cleavage of GATA-1. Nature. 2007;445(7123):102-5.
26. Verma A, Deb DK, Sassano A, Uddin S, Varga J, Wickrema A, et al. Activation of the p38 mitogen-activated protein kinase mediates the suppressive effects of type I interferons and transforming growth factor-beta on normal hematopoiesis. J Biol Chem. 2002;277(10):7726-35.
27. De Maria R, Zeuner A, Eramo A, Domenichelli C, Bonci D, Grignani F, et al. Negative regulation of erythropoiesis by caspase-mediated cleavage of GATA-1. Nature. 1999;401(6752):489-93.
28. Liu Y, Pop R, Sadegh C, Brugnara C, Haase VH, Socolovsky M. Suppression of Fas-FasL coexpression by erythropoietin mediates erythroblast expansion during the erythropoietic stress response in vivo. Blood. 2006;108(1):123-33.
29. Felli N, Pedini F, Zeuner A, Petrucci E, Testa U, Conticello C, et al. Multiple members of the TNF superfamily contribute to IFN-gamma-mediated inhibition of erythropoiesis. J Immunol. 2005;175(3):1464-72.
30. De Maria R, Testa U, Luchetti L, Zeuner A, Stassi G, Pelosi E, et al. Apoptotic role of Fas/Fas ligand system in the regulation of erythropoiesis. Blood. 1999;93(3):796-803.
31. Delabesse E, Ogilvy S, Chapman MA, Piltz SG, Gottgens B, Green AR. Transcriptional regulation of the SCL locus: identification of an enhancer that targets the primitive erythroid lineage in vivo. Mol Cell Biol. 2005;25(12):5215-25.
32. Zeuner A, Eramo A, Testa U, Felli N, Pelosi E, Mariani G, et al. Control of erythroid cell production via caspase-mediated cleavage of transcription factor SCL/Tal-1. Cell Death Differ. 2003;10(8):905-13.
33. Scaffidi C, Schmitz I, Krammer PH, Peter ME. The role of c-FLIP in modulation of CD95-induced apoptosis. J Biol Chem. 1999;274(3):1541-8.
34. Socolovsky M. Molecular insights into stress erythropoiesis. Curr Opin Hematol. 2007;14(3):215-24.
35. Silvestris F, Cafforio P, Tucci M, Dammacco F. Negative regulation of erythroblast maturation by Fas-L(+)/TRAIL(+) highly malignant plasma cells: a major pathogenetic mechanism of anemia in multiple myeloma. Blood. 2002;99(4):1305-13.
36. Perkins DL, Michaelson J, Marshak-Rothstein A. The lpr gene is associated with resistance to engraftment by lymphoid but not erythroid stem cells from normal mice. J Immunol. 1987;138(2):466-9.
37. Dai CH, Price JO, Brunner T, Krantz SB. Fas ligand is present in human erythroid colony-forming cells and interacts with Fas induced by interferon gamma to produce erythroid cell apoptosis. Blood. 1998;91(4):1235-42.
38. Wiley SR, Schooley K, Smolak PJ, Din WS, Huang CP, Nicholl JK, et al. Identification and characterization of a new member of the TNF family that induces apoptosis. Immunity. 1995;3(6):673-82.
39. Sheikh MS, Burns TF, Huang Y, Wu GS, Amundson S, Brooks KS, et al. p53-dependent and -independent regulation of the death receptor KILLER/DR5 gene expression in response to genotoxic stress and tumor necrosis factor alpha. Cancer Res. 1998;58(8):1593-8.
40. Herbeuval JP, Lambert C, Sabido O, Cottier M, Fournel P, Dy M, et al. Macrophages from cancer patients: analysis of TRAIL, TRAIL receptors, and colon tumor cell apoptosis. J Natl Cancer Inst. 2003;95(8):611-21.

41. Werner AB, de Vries E, Tait SW, Bontjer I, Borst J. TRAIL receptor and CD95 signal to mitochondria via FADD, caspase-8/10, Bid, and Bax but differentially regulate events downstream from truncated Bid. J Biol Chem. 2002;277(43):40760-7.
42. Sträter J, Möller P. TRAIL and viral infection. Vitam Horm. 2004;67:257-74.
43. Kayagaki N, Yamaguchi N, Nakayama M, Eto H, Okumura K, Yagita H. Type I interferons (IFNs) regulate tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) expression on human T cells: A novel mechanism for the antitumor effects of type I IFNs. J Exp Med. 1999;189(9):1451-60.
44. Morceau F, Dicato M, Diederich M. Pro-inflammatory cytokine-mediated anemia: regarding molecular mechanisms of erythropoiesis. Mediators Inflamm. 2009;2009:405016.
45. Zamai L, Secchiero P, Pierpaoli S, Bassini A, Papa S, Alnemri ES, et al. TNF-related apoptosis-inducing ligand (TRAIL) as a negative regulator of normal human erythropoiesis. Blood. 2000;95(12):3716-24.
46. Zauli G, Secchiero P. The role of the TRAIL/TRAIL receptors system in hematopoiesis and endothelial cell biology. Cytokine Growth Factor Rev. 2006;17(4):245-57.
47. Secchiero P, Melloni E, Heikinheimo M, Mannisto S, Di Pietro R, Iacone A, et al. TRAIL regulates normal erythroid maturation through an ERK-dependent pathway. Blood. 2004;103(2):517-22.
48. Secchiero P, Gonelli A, Mirandola P, Melloni E, Zamai L, Celeghini C, et al. Tumor necrosis factor-related apoptosis-inducing ligand induces monocytic maturation of leukemic and normal myeloid precursors through a caspase-dependent pathway. Blood. 2002;100(7):2421-9.
49. Lum JJ, Bren G, McClure R, Badley AD. Elimination of senescent neutrophils by TNF-related apoptosis-inducing [corrected] ligand. J Immunol. 2005;175(2):1232-8.
50. Oyaizu N, Adachi Y, Hashimoto F, McCloskey TW, Hosaka N, Kayagaki N, et al. Monocytes express Fas ligand upon CD4 cross-linking and induce CD4+ T cells apoptosis: a possible mechanism of bystander cell death in HIV infection. J Immunol. 1997;158(5):2456-63.
51. Koury MJ, Bondurant MC, Atkinson JB. Erythropoietin control of terminal erythroid differentiation: maintenance of cell viability, production of hemoglobin, and development of the erythrocyte membrane. Blood Cells. 1987;13(1-2):217-26.
52. Koury MJ, Bondurant MC. Erythropoietin retards DNA breakdown and prevents programmed death in erythroid progenitor cells. Science. 1990;248(4953):378-81.
53. Ogawa C, Tsuchiya K, Maeda K. Hypoxia-Inducible Factor Prolyl Hydroxylase Inhibitors and Iron Metabolism. Int J Mol Sci. 2023;24(3).
54. Blank U, Karlsson S. TGF-β signaling in the control of hematopoietic stem cells. Blood. 2015;125(23):3542-50.
55. Aoto M, Iwashita A, Mita K, Ohkubo N, Tsujimoto Y, Mitsuda N. Transferrin receptor 1 is required for enucleation of mouse erythroblasts during terminal differentiation. FEBS Open Bio. 2019;9(2):291-303.
56. Fouquet G, Coman T, Hermine O, Côté F. Serotonin, hematopoiesis and stem cells. Pharmacol Res. 2019;140:67-74.
57. Fouquet G, Rossignol J, Garcelon N, Hermine O, Côté F, Coman T. Targeting Serotonin With Common Antidepressants Induces Rapid Recovery From Cytopenia. Stem Cells Transl Med. 2022;11(9):927-31.
58. Miyake T, Kung CK, Goldwasser E. Purification of human erythropoietin. J Biol Chem. 1977;252(15):5558-64.
59. Lin FK, Suggs S, Lin CH, Browne JK, Smalling R, Egrie JC, et al. Cloning and expression of the human erythropoietin gene. Proc Natl Acad Sci U S A. 1985;82(22):7580-4.
60. Suzuki N. Erythropoietin gene expression: developmental-stage specificity, cell-type specificity, and hypoxia inducibility. Tohoku J Exp Med. 2015;235(3):233-40.
61. Hirano I, Suzuki N. The Neural Crest as the First Production Site of the Erythroid Growth Factor Erythropoietin. Front Cell Dev Biol. 2019;7:105.
62. Gruber M, Hu CJ, Johnson RS, Brown EJ, Keith B, Simon MC. Acute postnatal ablation of Hif-2alpha results in anemia. Proc Natl Acad Sci U S A. 2007;104(7):2301-6.

63. Haase VH. Regulation of erythropoiesis by hypoxia-inducible factors. Blood Rev. 2013;27(1):41-53.
64. Kobayashi H, Liu Q, Binns TC, Urrutia AA, Davidoff O, Kapitsinou PP, et al. Distinct subpopulations of FOXD1 stroma-derived cells regulate renal erythropoietin. J Clin Invest. 2016;126(5):1926-38.
65. Shih HM, Wu CJ, Lin SL. Physiology and pathophysiology of renal erythropoietin-producing cells. J Formos Med Assoc. 2018;117(11):955-63.
66. Fandrey J, Bunn HF. In vivo and in vitro regulation of erythropoietin mRNA: measurement by competitive polymerase chain reaction. Blood. 1993;81(3):617-23.
67. Masuda S, Okano M, Yamagishi K, Nagao M, Ueda M, Sasaki R. A novel site of erythropoietin production. Oxygen-dependent production in cultured rat astrocytes. J Biol Chem. 1994;269(30):19488-93.
68. Marti HH, Wenger RH, Rivas LA, Straumann U, Digicaylioglu M, Henn V, et al. Erythropoietin gene expression in human, monkey and murine brain. Eur J Neurosci. 1996;8(4):666-76.
69. Juul SE, Yachnis AT, Christensen RD. Tissue distribution of erythropoietin and erythropoietin receptor in the developing human fetus. Early Hum Dev. 1998;52(3):235-49.
70. Rankin EB, Wu C, Khatri R, Wilson TL, Andersen R, Araldi E, et al. The HIF signaling pathway in osteoblasts directly modulates erythropoiesis through the production of EPO. Cell. 2012;149(1):63-74.
71. Qin Q, Liu Y, Yang Z, Aimaijiang M, Ma R, Yang Y, et al. Hypoxia-Inducible Factors Signaling in Osteogenesis and Skeletal Repair. Int J Mol Sci. 2022;23(19).
72. Rankin EB, Biju MP, Liu Q, Unger TL, Rha J, Johnson RS, et al. Hypoxia-inducible factor-2 (HIF-2) regulates hepatic erythropoietin in vivo. J Clin Invest. 2007;117(4):1068-77.
73. Suzuki N, Gradin K, Poellinger L, Yamamoto M. Regulation of hypoxia-inducible gene expression after HIF activation. Exp Cell Res. 2017;356(2):182-6.
74. Jaakkola P, Mole DR, Tian YM, Wilson MI, Gielbert J, Gaskell SJ, et al. Targeting of HIF-alpha to the von Hippel-Lindau ubiquitylation complex by O2-regulated prolyl hydroxylation. Science. 2001;292(5516):468-72.
75. Semenza GL. Involvement of oxygen-sensing pathways in physiologic and pathologic erythropoiesis. Blood. 2009;114(10):2015-9.
76. Ohh M, Park CW, Ivan M, Hoffman MA, Kim TY, Huang LE, et al. Ubiquitination of hypoxia-inducible factor requires direct binding to the beta-domain of the von Hippel-Lindau protein. Nat Cell Biol. 2000;2(7):423-7.
77. Minamishima YA, Moslehi J, Bardeesy N, Cullen D, Bronson RT, Kaelin WG. Somatic inactivation of the PHD2 prolyl hydroxylase causes polycythemia and congestive heart failure. Blood. 2008;111(6):3236-44.
78. Ang SO, Chen H, Hirota K, Gordeuk VR, Jelinek J, Guan Y, et al. Disruption of oxygen homeostasis underlies congenital Chuvash polycythemia. Nat Genet. 2002;32(4):614-21.
79. Pastore YD, Jelinek J, Ang S, Guan Y, Liu E, Jedlickova K, et al. Mutations in the VHL gene in sporadic apparently congenital polycythemia. Blood. 2003;101(4):1591-5.
80. Percy MJ, Furlow PW, Lucas GS, Li X, Lappin TR, McMullin MF, et al. A gain-of-function mutation in the HIF2A gene in familial erythrocytosis. N Engl J Med. 2008;358(2):162-8.
81. Fandrey J. Oxygen-dependent and tissue-specific regulation of erythropoietin gene expression. Am J Physiol Regul Integr Comp Physiol. 2004;286(6):R977-88.
82. Dengler VL, Galbraith M, Espinosa JM. Transcriptional regulation by hypoxia inducible factors. Crit Rev Biochem Mol Biol. 2014;49(1):1-15.
83. Dames SA, Martinez-Yamout M, De Guzman RN, Dyson HJ, Wright PE. Structural basis for Hif-1 alpha /CBP recognition in the cellular hypoxic response. Proc Natl Acad Sci U S A. 2002;99(8):5271-6.
84. Freedman SJ, Sun ZY, Kung AL, France DS, Wagner G, Eck MJ. Structural basis for negative regulation of hypoxia-inducible factor-1alpha by CITED2. Nat Struct Biol. 2003;10(7):504-12.
85. Mahon PC, Hirota K, Semenza GL. FIH-1: a novel protein that interacts with HIF-1alpha and VHL to mediate repression of HIF-1 transcriptional activity. Genes Dev. 2001;15(20):2675-86.

86. Lappin TR, Lee FS. Update on mutations in the HIF: EPO pathway and their role in erythrocytosis. Blood Rev. 2019;37:100590.
87. Lisy K, Peet DJ. Turn me on: regulating HIF transcriptional activity. Cell Death Differ. 2008;15(4):642-9.
88. Jelkmann W. Regulation of erythropoietin production. J Physiol. 2011;589(Pt 6):1251-8.
89. Tomc J, Debeljak N. Molecular Insights into the Oxygen-Sensing Pathway and Erythropoietin Expression Regulation in Erythropoiesis. Int J Mol Sci. 2021;22(13).
90. Clements WK, Kim AD, Ong KG, Moore JC, Lawson ND, Traver D. A somitic Wnt16/Notch pathway specifies haematopoietic stem cells. Nature. 2011;474(7350):220-4.
91. Dzierzak E, Speck NA. Of lineage and legacy: the development of mammalian hematopoietic stem cells. Nat Immunol. 2008;9(2):129-36.
92. Varnum-Finney B, Purton LE, Yu M, Brashem-Stein C, Flowers D, Staats S, et al. The Notch ligand, Jagged-1, influences the development of primitive hematopoietic precursor cells. Blood. 1998;91(11):4084-91.
93. Duncan AW, Rattis FM, DiMascio LN, Congdon KL, Pazianos G, Zhao C, et al. Integration of Notch and Wnt signaling in hematopoietic stem cell maintenance. Nat Immunol. 2005;6(3):314-22.
94. Oh P, Lobry C, Gao J, Tikhonova A, Loizou E, Manet J, et al. In vivo mapping of notch pathway activity in normal and stress hematopoiesis. Cell Stem Cell. 2013;13(2):190-204.
95. Labat V, Bayard ENVT, Refeyton A, Huart M, Avalon M, Debeissat C, et al. Regulatory Crosstalk between Physiological Low O. Biomolecules. 2022;12(4).
96. Myllymäki MNM, Määttä J, Dimova EY, Izzi V, Väisänen T, Myllyharju J, et al. Notch Downregulation and Extramedullary Erythrocytosis in Hypoxia-Inducible Factor Prolyl 4-Hydroxylase 2-Deficient Mice. Mol Cell Biol. 2017;37(2).
97. Cosman D, Lyman SD, Idzerda RL, Beckmann MP, Park LS, Goodwin RG, et al. A new cytokine receptor superfamily. Trends Biochem Sci. 1990;15(7):265-70.
98. Liongue C, Sertori R, Ward AC. Evolution of Cytokine Receptor Signaling. J Immunol. 2016;197(1):11-8.
99. Suresh S, Rajvanshi PK, Noguchi CT. The Many Facets of Erythropoietin Physiologic and Metabolic Response. Front Physiol. 2019;10:1534.
100. Bhoopalan SV, Huang LJ, Weiss MJ. Erythropoietin regulation of red blood cell production: from bench to bedside and back. F1000Res. 2020;9.
101. Witthuhn BA, Quelle FW, Silvennoinen O, Yi T, Tang B, Miura O, et al. JAK2 associates with the erythropoietin receptor and is tyrosine phosphorylated and activated following stimulation with erythropoietin. Cell. 1993;74(2):227-36.
102. Tóthová Z, Tomc J, Debeljak N, Solár P. STAT5 as a Key Protein of Erythropoietin Signalization. Int J Mol Sci. 2021;22(13).
103. Socolovsky M, Nam H, Fleming MD, Haase VH, Brugnara C, Lodish HF. Ineffective erythropoiesis in Stat5a(-/-)5b(-/-) mice due to decreased survival of early erythroblasts. Blood. 2001;98(12):3261-73.
104. Smith MR, Satter LRF, Vargas-Hernández A. STAT5b: A master regulator of key biological pathways. Front Immunol. 2022;13:1025373.
105. Dev A, Byrne SM, Verma R, Ashton-Rickardt PG, Wojchowski DM. Erythropoietin-directed erythropoiesis depends on serpin inhibition of erythroblast lysosomal cathepsins. J Exp Med. 2013;210(2):225-32.
106. Zon LI, Youssoufian H, Mather C, Lodish HF, Orkin SH. Activation of the erythropoietin receptor promoter by transcription factor GATA-1. Proc Natl Acad Sci U S A. 1991;88(23):10638-41.
107. Paulson RF, Shi L, Wu DC. Stress erythropoiesis: new signals and new stress progenitor cells. Curr Opin Hematol. 2011;18(3):139-45.
108. Franke K, Kalucka J, Mamlouk S, Singh RP, Muschter A, Weidemann A, et al. HIF-1α is a protective factor in conditional PHD2-deficient mice suffering from severe HIF-2α-induced excessive erythropoiesis. Blood. 2013;121(8):1436-45.
109. Gassmann M, Muckenthaler MU. Adaptation of iron requirement to hypoxic conditions at high altitude. J Appl Physiol (1985). 2015;119(12):1432-40.

110. Iscove NN, Sieber F, Winterhalter KH. Erythroid colony formation in cultures of mouse and human bone marrow: analysis of the requirement for erythropoietin by gel filtration and affinity chromatography on agarose-concanavalin A. J Cell Physiol. 1974;83(2):309-20.
111. Wojchowski DM, Gregory RC, Miller CP, Pandit AK, Pircher TJ. Signal transduction in the erythropoietin receptor system. Exp Cell Res. 1999;253(1):143-56.
112. Casimir M, Colard M, Dussiot M, Roussel C, Martinez A, Peyssonnaux C, et al. Erythropoietin downregulates red blood cell clearance, increasing transfusion efficacy in severely anemic recipients. Am J Hematol. 2023;98(12):1923-33.
113. Naidech AM, Kahn MJ, Soong W, Green D, Batjer HH, Bleck TP. Packed red blood cell transfusion causes greater hemoglobin rise at a lower starting hemoglobin in patients with subarachnoid hemorrhage. Neurocrit Care. 2008;9(2):198-203.
114. Karafin MS, Bruhn R, Roubinian NH, Chowdhury D, Qu L, Snyder EL, et al. The impact of recipient factors on the lower-than-expected hemoglobin increment in transfused outpatients with hematologic diseases. Transfusion. 2019;59(8):2544-50.
115. Tsiftsoglou AS. Erythropoietin (EPO) as a Key Regulator of Erythropoiesis, Bone Remodeling and Endothelial Transdifferentiation of Multipotent Mesenchymal Stem Cells (MSCs): Implications in Regenerative Medicine. Cells. 2021;10(8).
116. Harris JM, Martin NE, Modi M. Pegylation: a novel process for modifying pharmacokinetics. Clin Pharmacokinet. 2001;40(7):539-51.
117. Boccia R, Lillie T, Tomita D, Balducci L. The effectiveness of darbepoetin alfa administered every 3 weeks on hematologic outcomes and quality of life in older patients with chemotherapy-induced anemia. Oncologist. 2007;12(5):584-93.
118. Ibbotson T, Goa KL. Darbepoetin alfa. Drugs. 2001;61(14):2097-104; discussion 105-6.
119. Saglimbene VM, Palmer SC, Ruospo M, Natale P, Craig JC, Strippoli GF. Continuous erythropoiesis receptor activator (CERA) for the anaemia of chronic kidney disease. Cochrane Database Syst Rev. 2017;8(8):CD009904.
120. Beguin Y, Loo M, R'Zik S, Sautois B, Lejeune F, Rorive G, et al. Quantitative assessment of erythropoiesis in haemodialysis patients demonstrates gradual expansion of erythroblasts during constant treatment with recombinant human erythropoietin. Br J Haematol. 1995;89(1):17-23.
121. Beguin Y, Loo M, R'Zik S, Sautois B, Lejeune F, Rorive G, et al. Early prediction of response to recombinant human erythropoietin in patients with the anemia of renal failure by serum transferrin receptor and fibrinogen. Blood. 1993;82(7):2010-6.
122. Locatelli F, Bárány P, Covic A, De Francisco A, Del Vecchio L, Goldsmith D, et al. Kidney Disease: Improving Global Outcomes guidelines on anaemia management in chronic kidney disease: a European Renal Best Practice position statement. Nephrol Dial Transplant. 2013;28(6):1346-59.
123. Beguin Y. Erythropoietin and the anemia of cancer. Acta Clin Belg. 1996;51(1):36-52.
124. Ferreira FA, Benites BD, Costa FF, Gilli S, Olalla-Saad ST. Recombinant erythropoietin as alternative to red cell transfusion in sickle cell disease. Vox Sang. 2019;114(2):178-81.
125. Hellström-Lindberg E, Ahlgren T, Beguin Y, Carlsson M, Carneskog J, Dahl IM, et al. Treatment of anemia in myelodysplastic syndromes with granulocyte colony-stimulating factor plus erythropoietin: results from a randomized phase II study and long-term follow-up of 71 patients. Blood. 1998;92(1):68-75.
126. Locatelli F, Zecca M, Beguin Y, Giorgiani G, Ponchio L, De Stefano P, et al. Accelerated erythroid repopulation with no stem-cell competition effect in children treated with recombinant human erythropoietin after allogeneic bone marrow transplantation. Br J Haematol. 1993;84(4):752-4.
127. Martí-Carvajal AJ, Solà I. Treatment for anemia in people with AIDS. Cochrane Database Syst Rev. 2007(1):CD004776.
128. Biesma DH, Van de Wiel A, Beguin Y, Kraaijenhagen RJ, Marx JJ. Erythropoietic activity and iron metabolism in autologous blood donors during recombinant human erythropoietin therapy. Eur J Clin Invest. 1994;24(6):426-32.
129. Weiss G, Ganz T, Goodnough LT. Anemia of inflammation. Blood. 2019;133(1):40-50.

130. Ludwig H, Aapro M, Bokemeyer C, Glaspy J, Hedenus M, Littlewood TJ, et al. A European patient record study on diagnosis and treatment of chemotherapy-induced anaemia. Support Care Cancer. 2014;22(8):2197-206.
131. Aapro M, Österborg A, Gascón P, Ludwig H, Beguin Y. Prevalence and management of cancer-related anaemia, iron deficiency and the specific role of i.v. iron. Ann Oncol. 2012;23(8):1954-62.
132. Weiss G, Schett G. Anaemia in inflammatory rheumatic diseases. Nat Rev Rheumatol. 2013;9(4):205-15.
133. Parikh A, Natarajan S, Lipsitz SR, Katz SD. Iron deficiency in community-dwelling US adults with self-reported heart failure in the National Health and Nutrition Examination Survey III: prevalence and associations with anemia and inflammation. Circ Heart Fail. 2011;4(5):599-606.
134. Nemeth E, Rivera S, Gabayan V, Keller C, Taudorf S, Pedersen BK, et al. IL-6 mediates hypoferremia of inflammation by inducing the synthesis of the iron regulatory hormone hepcidin. J Clin Invest. 2004;113(9):1271-6.
135. Verga Falzacappa MV, Vujic Spasic M, Kessler R, Stolte J, Hentze MW, Muckenthaler MU. STAT3 mediates hepatic hepcidin expression and its inflammatory stimulation. Blood. 2007;109(1):353-8.
136. Cazzola M, Ponchio L, de Benedetti F, Ravelli A, Rosti V, Beguin Y, et al. Defective iron supply for erythropoiesis and adequate endogenous erythropoietin production in the anemia associated with systemic-onset juvenile chronic arthritis. Blood. 1996;87(11):4824-30.
137. Beguin Y, Clemons GK, Pootrakul P, Fillet G. Quantitative assessment of erythropoiesis and functional classification of anemia based on measurements of serum transferrin receptor and erythropoietin. Blood. 1993;81(4):1067-76.
138. Macciò A, Madeddu C, Gramignano G, Mulas C, Tanca L, Cherchi MC, et al. The role of inflammation, iron, and nutritional status in cancer-related anemia: results of a large, prospective, observational study. Haematologica. 2015;100(1):124-32.
139. Beguin Y, Yerna M, Loo M, Weber M, Fillet G. Erythropoiesis in multiple myeloma: defective red cell production due to inappropriate erythropoietin production. Br J Haematol. 1992;82(4):648-53.
140. Beguin Y, Lampertz S, Bron D, Fillet G. Serum erythropoietin in chronic lymphocytic leukaemia. Br J Haematol. 1996;93(1):154-6.
141. Dowlati A, R'Zik S, Fillet G, Beguin Y. Anaemia of lung cancer is due to impaired erythroid marrow response to erythropoietin stimulation as well as relative inadequacy of erythropoietin production. Br J Haematol. 1997;97(2):297-9.
142. Cazzola M, Mercuriali F, Brugnara C. Use of recombinant human erythropoietin outside the setting of uremia. Blood. 1997;89(12):4248-67.
143. Beguin Y. Prediction of response and other improvements on the limitations of recombinant human erythropoietin therapy in anemic cancer patients. Haematologica. 2002;87(11):1209-21.
144. Cazzola M, Beguin Y, Kloczko J, Spicka I, Coiffier B. Once-weekly epoetin beta is highly effective in treating anaemic patients with lymphoproliferative malignancy and defective endogenous erythropoietin production. Br J Haematol. 2003;122(3):386-93.
145. Beguin Y. Prediction of response to optimize outcome of treatment with erythropoietin. Semin Oncol. 1998;25(3 Suppl 7):27-34.
146. Cazzola M, Ponchio L, Pedrotti C, Farina G, Cerani P, Lucotti C, et al. Prediction of response to recombinant human erythropoietin (rHuEpo) in anemia of malignancy. Haematologica. 1996;81(5):434-41.
147. Abdel-Razeq H, Hashem H. Recent update in the pathogenesis and treatment of chemotherapy and cancer induced anemia. Crit Rev Oncol Hematol. 2020;145:102837.
148. Beguin Y, Jaspers A. Iron sucrose - characteristics, efficacy and regulatory aspects of an established treatment of iron deficiency and iron-deficiency anemia in a broad range of therapeutic areas. Expert Opin Pharmacother. 2014;15(14):2087-103.
149. Aapro M, Beguin Y, Bokemeyer C, Dicato M, Gascón P, Glaspy J, et al. Management of anaemia and iron deficiency in patients with cancer: ESMO Clinical Practice Guidelines. Ann Oncol. 2018;29(Suppl 4):iv271.

150. Bohlius J, Schmidlin K, Brillant C, Schwarzer G, Trelle S, Seidenfeld J, et al. Erythropoietin or Darbepoetin for patients with cancer--meta-analysis based on individual patient data. Cochrane Database Syst Rev. 2009;2009(3):CD007303.
151. Tonia T, Mettler A, Robert N, Schwarzer G, Seidenfeld J, Weingart O, et al. Erythropoietin or darbepoetin for patients with cancer. Cochrane Database Syst Rev. 2012;12(12):CD003407.
152. Hedenus M, Osterborg A, Tomita D, Bohac C, Coiffier B. Effects of erythropoiesis-stimulating agents on survival and other outcomes in patients with lymphoproliferative malignancies: a study-level meta-analysis. Leuk Lymphoma. 2012;53(11):2151-8.
153. Ludwig H, Crawford J, Osterborg A, Vansteenkiste J, Henry DH, Fleishman A, et al. Pooled analysis of individual patient-level data from all randomized, double-blind, placebo-controlled trials of darbepoetin alfa in the treatment of patients with chemotherapy-induced anemia. J Clin Oncol. 2009;27(17):2838-47.
154. Aapro M, Osterwalder B, Scherhag A, Burger HU. Epoetin-beta treatment in patients with cancer chemotherapy-induced anaemia: the impact of initial haemoglobin and target haemoglobin levels on survival, tumour progression and thromboembolic events. Br J Cancer. 2009;101(12):1961-71.
155. Locatelli F, Fishbane S, Block GA, Macdougall IC. Targeting Hypoxia-Inducible Factors for the Treatment of Anemia in Chronic Kidney Disease Patients. Am J Nephrol. 2017;45(3):187-99.
156. Koury MJ, Agarwal R, Chertow GM, Eckardt KU, Fishbane S, Ganz T, et al. Erythropoietic effects of vadadustat in patients with anemia associated with chronic kidney disease. Am J Hematol. 2022;97(9):1178-88.
157. Ganz T, Locatelli F, Arici M, Akizawa T, Reusch M. Iron Parameters in Patients Treated with Roxadustat for Anemia of Chronic Kidney Disease. J Clin Med. 2023;12(13).
158. Yang J, Xing J, Zhu X, Xie X, Wang L, Zhang X. Effects of hypoxia-inducible factor-prolyl hydroxylase inhibitors. Front Endocrinol (Lausanne). 2023;14:1131516.
159. Sugahara M, Tanaka T, Nangaku M. Prolyl hydroxylase domain inhibitors as a novel therapeutic approach against anemia in chronic kidney disease. Kidney Int. 2017;92(2):306-12.
160. Nishide S, Uchida J, Matsunaga S, Tokudome K, Yamaguchi T, Kabei K, et al. Prolyl-hydroxylase inhibitors reconstitute tumor blood vessels in mice. J Pharmacol Sci. 2020;143(2):122-6.
161. Gaete D, Rodriguez D, Watts D, Sormendi S, Chavakis T, Wielockx B. HIF-Prolyl Hydroxylase Domain Proteins (PHDs) in Cancer-Potential Targets for Anti-Tumor Therapy? Cancers (Basel). 2021;13(5).
162. Henry DH, Glaspy J, Harrup R, Mittelman M, Zhou A, Carraway HE, et al. Roxadustat for the treatment of anemia in patients with lower-risk myelodysplastic syndrome: Open-label, dose-selection, lead-in stage of a phase 3 study. Am J Hematol. 2022;97(2):174-84.
163. Bataller A, Montalban-Bravo G, Soltysiak KA, Garcia-Manero G. The role of TGFβ in hematopoiesis and myeloid disorders. Leukemia. 2019;33(5):1076-89.
164. Zhou L, Nguyen AN, Sohal D, Ying Ma J, Pahanish P, Gundabolu K, et al. Inhibition of the TGF-beta receptor I kinase promotes hematopoiesis in MDS. Blood. 2008;112(8):3434-43.
165. Delgado J, Voltz C, Stain M, Balkowiec-Iskra E, Mueller B, Wernsperger J, et al. The European Medicines Agency Review of Luspatercept for the Treatment of Adult Patients With Transfusion-dependent Anemia Caused by Low-risk Myelodysplastic Syndromes With Ring Sideroblasts or Beta-thalassemia. Hemasphere. 2021;5(8):e616.
166. Kang C, Syed YY. Luspatercept: A Review in Transfusion-Dependent Anaemia due to Myelodysplastic Syndromes or β-Thalassaemia. Drugs. 2021;81(8):945-52.
167. Allampallam K, Shetty V, Mundle S, Dutt D, Kravitz H, Reddy PL, et al. Biological significance of proliferation, apoptosis, cytokines, and monocyte/macrophage cells in bone marrow biopsies of 145 patients with myelodysplastic syndrome. Int J Hematol. 2002;75(3):289-97.
168. Blank U, Karlsson S. The role of Smad signaling in hematopoiesis and translational hematology. Leukemia. 2011;25(9):1379-88.
169. Attie KM, Allison MJ, McClure T, Boyd IE, Wilson DM, Pearsall AE, et al. A phase 1 study of ACE-536, a regulator of erythroid differentiation, in healthy volunteers. Am J Hematol. 2014;89(7):766-70.

170. Fenaux P, Platzbecker U, Mufti GJ, Garcia-Manero G, Buckstein R, Santini V, et al. Luspatercept in Patients with Lower-Risk Myelodysplastic Syndromes. N Engl J Med. 2020;382(2):140-51.
171. Fenaux P, Kiladjian JJ, Platzbecker U. Luspatercept for the treatment of anemia in myelodysplastic syndromes and primary myelofibrosis. Blood. 2019;133(8):790-4.
172. Ceglia I, Dueck AC, Masiello F, Martelli F, He W, Federici G, et al. Preclinical rationale for TGF-β inhibition as a therapeutic target for the treatment of myelofibrosis. Exp Hematol. 2016;44(12):1138-55.e4.
173. Cappellini MD, Viprakasit V, Taher AT, Georgiev P, Kuo KHM, Coates T, et al. A Phase 3 Trial of Luspatercept in Patients with Transfusion-Dependent β-Thalassemia. N Engl J Med. 2020;382(13):1219-31.
174. Melchiori L, Gardenghi S, Rivella S. beta-Thalassemia: HiJAKing Ineffective Erythropoiesis and Iron Overload. Adv Hematol. 2010;2010:938640.
175. Suragani RN, Cawley SM, Li R, Wallner S, Alexander MJ, Mulivor AW, et al. Modified activin receptor IIB ligand trap mitigates ineffective erythropoiesis and disease complications in murine β-thalassemia. Blood. 2014;123(25):3864-72.
176. Suragani RN, Cadena SM, Cawley SM, Sako D, Mitchell D, Li R, et al. Transforming growth factor-β superfamily ligand trap ACE-536 corrects anemia by promoting late-stage erythropoiesis. Nat Med. 2014;20(4):408-14.
177. Piga A, Perrotta S, Gamberini MR, Voskaridou E, Melpignano A, Filosa A, et al. Luspatercept improves hemoglobin levels and blood transfusion requirements in a study of patients with β-thalassemia. Blood. 2019;133(12):1279-89.
178. Musallam KM, Taher AT, Kattamis A, Kuo KHM, Sheth S, Cappellini MD. Profile of Luspatercept in the Treatment of Anemia in Adults with Non-Transfusion-Dependent β-Thalassemia (NTDT): Design, Development and Potential Place in Therapy. Drug Des Devel Ther. 2023;17:1583-91.
179. Frey PA, Reed GH. The ubiquity of iron. ACS Chem Biol. 2012;7(9):1477-81.
180. Andrews NC. Disorders of iron metabolism. N Engl J Med. 1999;341(26):1986-95.
181. Pantopoulos K, Porwal SK, Tartakoff A, Devireddy L. Mechanisms of mammalian iron homeostasis. Biochemistry. 2012;51(29):5705-24.
182. Fujita H. Molecular mechanism of heme biosynthesis. Tohoku J Exp Med. 1997;183(2):83-99.
183. Guengerich FP, Waterman MR, Egli M. Recent Structural Insights into Cytochrome P450 Function. Trends Pharmacol Sci. 2016;37(8):625-40.
184. Oppenheim EW, Nasrallah IM, Mastri MG, Stover PJ. Mimosine is a cell-specific antagonist of folate metabolism. J Biol Chem. 2000;275(25):19268-74.
185. Lederman HM, Cohen A, Lee JW, Freedman MH, Gelfand EW. Deferoxamine: a reversible S-phase inhibitor of human lymphocyte proliferation. Blood. 1984;64(3):748-53.
186. Zhang C. Essential functions of iron-requiring proteins in DNA replication, repair and cell cycle control. Protein Cell. 2014;5(10):750-60.
187. Le NT, Richardson DR. The role of iron in cell cycle progression and the proliferation of neoplastic cells. Biochim Biophys Acta. 2002;1603(1):31-46.
188. Dixon SJ, Lemberg KM, Lamprecht MR, Skouta R, Zaitsev EM, Gleason CE, et al. Ferroptosis: an iron-dependent form of nonapoptotic cell death. Cell. 2012;149(5):1060-72.
189. Tang D, Chen X, Kang R, Kroemer G. Ferroptosis: molecular mechanisms and health implications. Cell Res. 2021;31(2):107-25.
190. Cao JY, Dixon SJ. Mechanisms of ferroptosis. Cell Mol Life Sci. 2016;73(11-12):2195-209.
191. Friedmann Angeli JP, Schneider M, Proneth B, Tyurina YY, Tyurin VA, Hammond VJ, et al. Inactivation of the ferroptosis regulator Gpx4 triggers acute renal failure in mice. Nat Cell Biol. 2014;16(12):1180-91.
192. Skouta R, Dixon SJ, Wang J, Dunn DE, Orman M, Shimada K, et al. Ferrostatins inhibit oxidative lipid damage and cell death in diverse disease models. J Am Chem Soc. 2014;136(12):4551-6.
193. Xie Y, Hou W, Song X, Yu Y, Huang J, Sun X, et al. Ferroptosis: process and function. Cell Death Differ. 2016;23(3):369-79.

194. Song R, Wang R, Shen Z, Chu H. Sevoflurane diminishes neurogenesis and promotes ferroptosis in embryonic prefrontal cortex via inhibiting nuclear factor-erythroid 2-related factor 2 expression. Neuroreport. 2022;33(6):252-8.
195. Zhu L, Luo S, Zhu Y, Tang S, Li C, Jin X, et al. The Emerging Role of Ferroptosis in Various Chronic Liver Diseases: Opportunity or Challenge. J Inflamm Res. 2023;16:381-9.
196. Koppenol WH. The Haber-Weiss cycle--70 years later. Redox Rep. 2001;6(4):229-34.
197. Birben E, Sahiner UM, Sackesen C, Erzurum S, Kalayci O. Oxidative stress and antioxidant defense. World Allergy Organ J. 2012;5(1):9-19.
198. Bullen JJ, Rogers HJ, Spalding PB, Ward CG. Iron and infection: the heart of the matter. FEMS Immunol Med Microbiol. 2005;43(3):325-30.
199. Hentze MW, Muckenthaler MU, Galy B, Camaschella C. Two to tango: regulation of Mammalian iron metabolism. Cell. 2010;142(1):24-38.
200. Hunt JR, Zito CA, Johnson LK. Body iron excretion by healthy men and women. Am J Clin Nutr. 2009;89(6):1792-8.
201. GIBLETT ER, COLEMAN DH, PIRZIOBIROLI G, DONOHUE DM, MOTULSKY AG, FINCH CA. Erythrokinetics: quantitative measurements of red cell production and destruction in normal subjects and patients with anemia. Blood. 1956;11(4):291-309.
202. Borges MD, Sesti-Costa R. Macrophages: key players in erythrocyte turnover. Hematol Transfus Cell Ther. 2022;44(4):574-81.
203. Vallelian F, Buehler PW, Schaer DJ. Hemolysis, free hemoglobin toxicity, and scavenger protein therapeutics. Blood. 2022;140(17):1837-44.
204. Knutson MD. Iron transport proteins: Gateways of cellular and systemic iron homeostasis. J Biol Chem. 2017;292(31):12735-43.
205. Gunshin H, Starr CN, Direnzo C, Fleming MD, Jin J, Greer EL, et al. Cybrd1 (duodenal cytochrome b) is not necessary for dietary iron absorption in mice. Blood. 2005;106(8):2879-83.
206. McKie AT, Barrow D, Latunde-Dada GO, Rolfs A, Sager G, Mudaly E, et al. An iron-regulated ferric reductase associated with the absorption of dietary iron. Science. 2001;291(5509):1755-9.
207. Gunshin H, Fujiwara Y, Custodio AO, Direnzo C, Robine S, Andrews NC. Slc11a2 is required for intestinal iron absorption and erythropoiesis but dispensable in placenta and liver. J Clin Invest. 2005;115(5):1258-66.
208. Fleming MD, Romano MA, Su MA, Garrick LM, Garrick MD, Andrews NC. Nramp2 is mutated in the anemic Belgrade (b) rat: evidence of a role for Nramp2 in endosomal iron transport. Proc Natl Acad Sci U S A. 1998;95(3):1148-53.
209. Fleming MD, Trenor CC, Su MA, Foernzler D, Beier DR, Dietrich WF, et al. Microcytic anaemia mice have a mutation in Nramp2, a candidate iron transporter gene. Nat Genet. 1997;16(4):383-6.
210. Mims MP, Guan Y, Pospisilova D, Priwitzerova M, Indrak K, Ponka P, et al. Identification of a human mutation of DMT1 in a patient with microcytic anemia and iron overload. Blood. 2005;105(3):1337-42.
211. Tenhunen R, Gräsbeck R, Kouvonen I, Lundberg M. An intestinal receptor for heme: its parital characterization. Int J Biochem. 1980;12(5-6):713-6.
212. Shayeghi M, Latunde-Dada GO, Oakhill JS, Laftah AH, Takeuchi K, Halliday N, et al. Identification of an intestinal heme transporter. Cell. 2005;122(5):789-801.
213. White C, Yuan X, Schmidt PJ, Bresciani E, Samuel TK, Campagna D, et al. HRG1 is essential for heme transport from the phagolysosome of macrophages during erythrophagocytosis. Cell Metab. 2013;17(2):261-70.
214. West AR, Oates PS. Subcellular location of heme oxygenase 1 and 2 and divalent metal transporter 1 in relation to endocytotic markers during heme iron absorption. J Gastroenterol Hepatol. 2008;23(1):150-8.
215. Rajagopal A, Rao AU, Amigo J, Tian M, Upadhyay SK, Hall C, et al. Haem homeostasis is regulated by the conserved and concerted functions of HRG-1 proteins. Nature. 2008;453(7198):1127-31.

216. Donovan A, Brownlie A, Zhou Y, Shepard J, Pratt SJ, Moynihan J, et al. Positional cloning of zebrafish ferroportin1 identifies a conserved vertebrate iron exporter. Nature. 2000;403(6771):776-81.
217. Philpott CC, Ryu MS, Frey A, Patel S. Cytosolic iron chaperones: Proteins delivering iron cofactors in the cytosol of mammalian cells. J Biol Chem. 2017;292(31):12764-71.
218. McKie AT, Marciani P, Rolfs A, Brennan K, Wehr K, Barrow D, et al. A novel duodenal iron-regulated transporter, IREG1, implicated in the basolateral transfer of iron to the circulation. Mol Cell. 2000;5(2):299-309.
219. Abboud S, Haile DJ. A novel mammalian iron-regulated protein involved in intracellular iron metabolism. J Biol Chem. 2000;275(26):19906-12.
220. Donovan A, Lima CA, Pinkus JL, Pinkus GS, Zon LI, Robine S, et al. The iron exporter ferroportin/Slc40a1 is essential for iron homeostasis. Cell Metab. 2005;1(3):191-200.
221. Mitchell CJ, Shawki A, Ganz T, Nemeth E, Mackenzie B. Functional properties of human ferroportin, a cellular iron exporter reactive also with cobalt and zinc. Am J Physiol Cell Physiol. 2014;306(5):C450-9.
222. Vashchenko G, Macgillivray RT. Functional role of the putative iron ligands in the ferroxidase activity of recombinant human hephaestin. J Biol Inorg Chem. 2012;17(8):1187-95.
223. Ohgami RS, Campagna DR, Greer EL, Antiochos B, McDonald A, Chen J, et al. Identification of a ferrireductase required for efficient transferrin-dependent iron uptake in erythroid cells. Nat Genet. 2005;37(11):1264-9.
224. Iwai K. Regulation of cellular iron metabolism: Iron-dependent degradation of IRP by SCF. Free Radic Biol Med. 2019;133:64-8.
225. Chiou B, Connor JR. Emerging and Dynamic Biomedical Uses of Ferritin. Pharmaceuticals (Basel). 2018;11(4).
226. Arosio P, Ingrassia R, Cavadini P. Ferritins: a family of molecules for iron storage, antioxidation and more. Biochim Biophys Acta. 2009;1790(7):589-99.
227. Wang Z, Li C, Ellenburg M, Soistman E, Ruble J, Wright B, et al. Structure of human ferritin L chain. Acta Crystallogr D Biol Crystallogr. 2006;62(Pt 7):800-6.
228. Santambrogio P, Levi S, Cozzi A, Corsi B, Arosio P. Evidence that the specificity of iron incorporation into homopolymers of human ferritin L- and H-chains is conferred by the nucleation and ferroxidase centres. Biochem J. 1996;314 ( Pt 1)(Pt 1):139-44.
229. Cohen LA, Gutierrez L, Weiss A, Leichtmann-Bardoogo Y, Zhang DL, Crooks DR, et al. Serum ferritin is derived primarily from macrophages through a nonclassical secretory pathway. Blood. 2010;116(9):1574-84.
230. Ferreira C, Bucchini D, Martin ME, Levi S, Arosio P, Grandchamp B, et al. Early embryonic lethality of H ferritin gene deletion in mice. J Biol Chem. 2000;275(5):3021-4.
231. Thompson K, Menzies S, Muckenthaler M, Torti FM, Wood T, Torti SV, et al. Mouse brains deficient in H-ferritin have normal iron concentration but a protein profile of iron deficiency and increased evidence of oxidative stress. J Neurosci Res. 2003;71(1):46-63.
232. Clough G. The steady-state transport of cationized ferritin by endothelial cell vesicles. J Physiol. 1982;328:389-401.
233. Levi S, Corsi B, Bosisio M, Invernizzi R, Volz A, Sanford D, et al. A human mitochondrial ferritin encoded by an intronless gene. J Biol Chem. 2001;276(27):24437-40.
234. Levi S, Ripamonti M, Dardi M, Cozzi A, Santambrogio P. Mitochondrial Ferritin: Its Role in Physiological and Pathological Conditions. Cells. 2021;10(8).
235. Ghosh S, Hevi S, Chuck SL. Regulated secretion of glycosylated human ferritin from hepatocytes. Blood. 2004;103(6):2369-76.
236. Fan Y, Yamada T, Shimizu T, Nanashima N, Akita M, Suto K, et al. Ferritin expression in rat hepatocytes and Kupffer cells after lead nitrate treatment. Toxicol Pathol. 2009;37(2):209-17.
237. Wesselius LJ, Nelson ME, Skikne BS. Increased release of ferritin and iron by iron-loaded alveolar macrophages in cigarette smokers. Am J Respir Crit Care Med. 1994;150(3):690-5.

238. Mancias JD, Wang X, Gygi SP, Harper JW, Kimmelman AC. Quantitative proteomics identifies NCOA4 as the cargo receptor mediating ferritinophagy. Nature. 2014;509(7498):105-9.
239. Mancias JD, Pontano Vaites L, Nissim S, Biancur DE, Kim AJ, Wang X, et al. Ferritinophagy via NCOA4 is required for erythropoiesis and is regulated by iron dependent HERC2-mediated proteolysis. Elife. 2015;4.
240. Truman-Rosentsvit M, Berenbaum D, Spektor L, Cohen LA, Belizowsky-Moshe S, Lifshitz L, et al. Ferritin is secreted via 2 distinct nonclassical vesicular pathways. Blood. 2018;131(3):342-52.
241. Jacobs A, Miller F, Worwood M, Beamish MR, Wardrop CA. Ferritin in the serum of normal subjects and patients with iron deficiency and iron overload. Br Med J. 1972;4(5834):206-8.
242. Walters GO, Miller FM, Worwood M. Serum ferritin concentration and iron stores in normal subjects. J Clin Pathol. 1973;26(10):770-2.
243. Saito H. METABOLISM OF IRON STORES. Nagoya J Med Sci. 2014;76(3-4):235-54.
244. Iancu TC. Ferritin and hemosiderin in pathological tissues. Electron Microsc Rev. 1992;5(2):209-29.
245. Richter GW. The iron-loaded cell--the cytopathology of iron storage. A review. Am J Pathol. 1978;91(2):362-404.
246. Archiv für Pathologische Anatomie und Physiologie und für Klinische Medicin. Br Foreign Med Chir Rev. 1861;27(53):52-65.
247. Iancu TC. Biological and ultrastructural aspects of iron overload: an overview. Pediatr Pathol. 1990;10(1-2):281-96.
248. Theil EC. The IRE (iron regulatory element) family: structures which regulate mRNA translation or stability. Biofactors. 1993;4(2):87-93.
249. Kühn LC. Iron regulatory proteins and their role in controlling iron metabolism. Metallomics. 2015;7(2):232-43.
250. Salahudeen AA, Thompson JW, Ruiz JC, Ma HW, Kinch LN, Li Q, et al. An E3 ligase possessing an iron-responsive hemerythrin domain is a regulator of iron homeostasis. Science. 2009;326(5953):722-6.
251. Theil EC, McKenzie RA, Sierzputowska-Gracz H. Structure and function of IREs, the noncoding mRNA sequences regulating synthesis of ferritin, transferrin receptor and (erythroid) 5-aminolevulinate synthase. Adv Exp Med Biol. 1994;356:111-8.
252. Sanchez M, Galy B, Muckenthaler MU, Hentze MW. Iron-regulatory proteins limit hypoxia-inducible factor-2alpha expression in iron deficiency. Nat Struct Mol Biol. 2007;14(5):420-6.
253. Christova T, Templeton DM. Effect of hypoxia on the binding and subcellular distribution of iron regulatory proteins. Mol Cell Biochem. 2007;301(1-2):21-32.
254. Sonnweber T, Nachbaur D, Schroll A, Nairz M, Seifert M, Demetz E, et al. Hypoxia induced downregulation of hepcidin is mediated by platelet derived growth factor BB. Gut. 2014;63(12):1951-9.
255. Mastrogiannaki M, Matak P, Mathieu JR, Delga S, Mayeux P, Vaulont S, et al. Hepatic hypoxia-inducible factor-2 down-regulates hepcidin expression in mice through an erythropoietin-mediated increase in erythropoiesis. Haematologica. 2012;97(6):827-34.
256. Taylor M, Qu A, Anderson ER, Matsubara T, Martin A, Gonzalez FJ, et al. Hypoxia-inducible factor-2α mediates the adaptive increase of intestinal ferroportin during iron deficiency in mice. Gastroenterology. 2011;140(7):2044-55.
257. Shah YM, Matsubara T, Ito S, Yim SH, Gonzalez FJ. Intestinal hypoxia-inducible transcription factors are essential for iron absorption following iron deficiency. Cell Metab. 2009;9(2):152-64.
258. Mastrogiannaki M, Matak P, Delga S, Deschemin JC, Vaulont S, Peyssonnaux C. Deletion of HIF-2α in the enterocytes decreases the severity of tissue iron loading in hepcidin knockout mice. Blood. 2012;119(2):587-90.
259. Lee PJ, Jiang BH, Chin BY, Iyer NV, Alam J, Semenza GL, et al. Hypoxia-inducible factor-1 mediates transcriptional activation of the heme oxygenase-1 gene in response to hypoxia. J Biol Chem. 1997;272(9):5375-81.

260. Mukhopadhyay CK, Mazumder B, Fox PL. Role of hypoxia-inducible factor-1 in transcriptional activation of ceruloplasmin by iron deficiency. J Biol Chem. 2000;275(28):21048-54.
261. Rolfs A, Kvietikova I, Gassmann M, Wenger RH. Oxygen-regulated transferrin expression is mediated by hypoxia-inducible factor-1. J Biol Chem. 1997;272(32):20055-62.
262. Lok CN, Ponka P. Identification of a hypoxia response element in the transferrin receptor gene. J Biol Chem. 1999;274(34):24147-52.
263. Li X, Lozovatsky L, Sukumaran A, Gonzalez L, Jain A, Liu D, et al. NCOA4 is regulated by HIF and mediates mobilization of murine hepatic iron stores after blood loss. Blood. 2020;136(23):2691-702.
264. Hahn PF, Bale WF, Ross JF, Balfour WM, Whipple GH. RADIOACTIVE IRON ABSORPTION BY GASTRO-INTESTINAL TRACT : INFLUENCE OF ANEMIA, ANOXIA, AND ANTECEDENT FEEDING DISTRIBUTION IN GROWING DOGS. J Exp Med. 1943;78(3):169-88.
265. Vanoaica L, Darshan D, Richman L, Schümann K, Kühn LC. Intestinal ferritin H is required for an accurate control of iron absorption. Cell Metab. 2010;12(3):273-82.
266. Andrews NC. Ferrit(in)ing out new mechanisms in iron homeostasis. Cell Metab. 2010;12(3):203-4.
267. Galy B, Ferring-Appel D, Becker C, Gretz N, Gröne HJ, Schümann K, et al. Iron regulatory proteins control a mucosal block to intestinal iron absorption. Cell Rep. 2013;3(3):844-57.
268. Ganz T. Hepcidin and iron regulation, 10 years later. Blood. 2011;117(17):4425-33.
269. Park CH, Valore EV, Waring AJ, Ganz T. Hepcidin, a urinary antimicrobial peptide synthesized in the liver. J Biol Chem. 2001;276(11):7806-10.
270. Krause A, Neitz S, Mägert HJ, Schulz A, Forssmann WG, Schulz-Knappe P, et al. LEAP-1, a novel highly disulfide-bonded human peptide, exhibits antimicrobial activity. FEBS Lett. 2000;480(2-3):147-50.
271. Lou DQ, Nicolas G, Lesbordes JC, Viatte L, Grimber G, Szajnert MF, et al. Functional differences between hepcidin 1 and 2 in transgenic mice. Blood. 2004;103(7):2816-21.
272. Pigeon C, Ilyin G, Courselaud B, Leroyer P, Turlin B, Brissot P, et al. A new mouse liver-specific gene, encoding a protein homologous to human antimicrobial peptide hepcidin, is overexpressed during iron overload. J Biol Chem. 2001;276(11):7811-9.
273. Nicolas G, Bennoun M, Devaux I, Beaumont C, Grandchamp B, Kahn A, et al. Lack of hepcidin gene expression and severe tissue iron overload in upstream stimulatory factor 2 (USF2) knockout mice. Proc Natl Acad Sci U S A. 2001;98(15):8780-5.
274. Nicolas G, Bennoun M, Porteu A, Mativet S, Beaumont C, Grandchamp B, et al. Severe iron deficiency anemia in transgenic mice expressing liver hepcidin. Proc Natl Acad Sci U S A. 2002;99(7):4596-601.
275. Roetto A, Papanikolaou G, Politou M, Alberti F, Girelli D, Christakis J, et al. Mutant antimicrobial peptide hepcidin is associated with severe juvenile hemochromatosis. Nat Genet. 2003;33(1):21-2.
276. Lesbordes-Brion JC, Viatte L, Bennoun M, Lou DQ, Ramey G, Houbron C, et al. Targeted disruption of the hepcidin 1 gene results in severe hemochromatosis. Blood. 2006;108(4):1402-5.
277. Valore EV, Ganz T. Posttranslational processing of hepcidin in human hepatocytes is mediated by the prohormone convertase furin. Blood Cells Mol Dis. 2008;40(1):132-8.
278. Peters HP, Laarakkers CM, Pickkers P, Masereeuw R, Boerman OC, Eek A, et al. Tubular reabsorption and local production of urine hepcidin-25. BMC Nephrol. 2013;14:70.
279. van Swelm RP, Wetzels JF, Verweij VG, Laarakkers CM, Pertijs JC, van der Wijst J, et al. Renal Handling of Circulating and Renal-Synthesized Hepcidin and Its Protective Effects against Hemoglobin-Mediated Kidney Injury. J Am Soc Nephrol. 2016;27(9):2720-32.
280. Nemeth E, Tuttle MS, Powelson J, Vaughn MB, Donovan A, Ward DM, et al. Hepcidin regulates cellular iron efflux by binding to ferroportin and inducing its internalization. Science. 2004;306(5704):2090-3.
281. McKie AT, Barlow DJ. The SLC40 basolateral iron transporter family (IREG1/ferroportin/MTP1). Pflugers Arch. 2004;447(5):801-6.

282. Delaby C, Pilard N, Gonçalves AS, Beaumont C, Canonne-Hergaux F. Presence of the iron exporter ferroportin at the plasma membrane of macrophages is enhanced by iron loading and down-regulated by hepcidin. Blood. 2005;106(12):3979-84.
283. Qiao B, Sugianto P, Fung E, Del-Castillo-Rueda A, Moran-Jimenez MJ, Ganz T, et al. Hepcidin-induced endocytosis of ferroportin is dependent on ferroportin ubiquitination. Cell Metab. 2012;15(6):918-24.
284. Aschemeyer S, Qiao B, Stefanova D, Valore EV, Sek AC, Ruwe TA, et al. Structure-function analysis of ferroportin defines the binding site and an alternative mechanism of action of hepcidin. Blood. 2018;131(8):899-910.
285. Vela D. Balance of cardiac and systemic hepcidin and its role in heart physiology and pathology. Lab Invest. 2018;98(3):315-26.
286. Kulaksiz H, Theilig F, Bachmann S, Gehrke SG, Rost D, Janetzko A, et al. The iron-regulatory peptide hormone hepcidin: expression and cellular localization in the mammalian kidney. J Endocrinol. 2005;184(2):361-70.
287. Theurl M, Theurl I, Hochegger K, Obrist P, Subramaniam N, van Rooijen N, et al. Kupffer cells modulate iron homeostasis in mice via regulation of hepcidin expression. J Mol Med (Berl). 2008;86(7):825-35.
288. Vela D. Hepcidin, an emerging and important player in brain iron homeostasis. J Transl Med. 2018;16(1):25.
289. Daher R, Lefebvre T, Puy H, Karim Z. Extrahepatic hepcidin production: The intriguing outcomes of recent years. World J Clin Cases. 2019;7(15):1926-36.
290. Merle U, Fein E, Gehrke SG, Stremmel W, Kulaksiz H. The iron regulatory peptide hepcidin is expressed in the heart and regulated by hypoxia and inflammation. Endocrinology. 2007;148(6):2663-8.
291. Lakhal-Littleton S, Wolna M, Chung YJ, Christian HC, Heather LC, Brescia M, et al. An essential cell-autonomous role for hepcidin in cardiac iron homeostasis. Elife. 2016;5.
292. Houamel D, Ducrot N, Lefebvre T, Daher R, Moulouel B, Sari MA, et al. Hepcidin as a Major Component of Renal Antibacterial Defenses against Uropathogenic Escherichia coli. J Am Soc Nephrol. 2016;27(3):835-46.
293. Wolff NA, Liu W, Fenton RA, Lee WK, Thévenod F, Smith CP. Ferroportin 1 is expressed basolaterally in rat kidney proximal tubule cells and iron excess increases its membrane trafficking. J Cell Mol Med. 2011;15(2):209-19.
294. Ferguson CJ, Wareing M, Ward DT, Green R, Smith CP, Riccardi D. Cellular localization of divalent metal transporter DMT-1 in rat kidney. Am J Physiol Renal Physiol. 2001;280(5):F803-14.
295. Kulaksiz H, Fein E, Redecker P, Stremmel W, Adler G, Cetin Y. Pancreatic beta-cells express hepcidin, an iron-uptake regulatory peptide. J Endocrinol. 2008;197(2):241-9.
296. Lunova M, Schwarz P, Nuraldeen R, Levada K, Kuscuoglu D, Stützle M, et al. Hepcidin knockout mice spontaneously develop chronic pancreatitis owing to cytoplasmic iron overload in acinar cells. J Pathol. 2017;241(1):104-14.
297. Cooksey RC, Jones D, Gabrielsen S, Huang J, Simcox JA, Luo B, et al. Dietary iron restriction or iron chelation protects from diabetes and loss of beta-cell function in the obese (ob/ob lep-/-) mouse. Am J Physiol Endocrinol Metab. 2010;298(6):E1236-43.
298. Hansen JB, Tonnesen MF, Madsen AN, Hagedorn PH, Friberg J, Grunnet LG, et al. Divalent metal transporter 1 regulates iron-mediated ROS and pancreatic β cell fate in response to cytokines. Cell Metab. 2012;16(4):449-61.
299. Bekri S, Gual P, Anty R, Luciani N, Dahman M, Ramesh B, et al. Increased adipose tissue expression of hepcidin in severe obesity is independent from diabetes and NASH. Gastroenterology. 2006;131(3):788-96.
300. Sal E, Yenicesu I, Celik N, Pasaoglu H, Celik B, Pasaoglu OT, et al. Relationship between obesity and iron deficiency anemia: is there a role of hepcidin? Hematology. 2018;23(8):542-8.
301. Liu XB, Nguyen NB, Marquess KD, Yang F, Haile DJ. Regulation of hepcidin and ferroportin expression by lipopolysaccharide in splenic macrophages. Blood Cells Mol Dis. 2005;35(1):47-56.

302. Zechel S, Huber-Wittmer K, von Bohlen und Halbach O. Distribution of the iron-regulating protein hepcidin in the murine central nervous system. J Neurosci Res. 2006;84(4):790-800.
303. Schwarz P, Kübler JA, Strnad P, Müller K, Barth TF, Gerloff A, et al. Hepcidin is localised in gastric parietal cells, regulates acid secretion and is induced by Helicobacter pylori infection. Gut. 2012;61(2):193-201.
304. Chen QX, Song SW, Chen QH, Zeng CL, Zheng X, Wang JL, et al. Silencing airway epithelial cell-derived hepcidin exacerbates sepsis induced acute lung injury. Crit Care. 2014;18(4):470.
305. Tesfay L, Clausen KA, Kim JW, Hegde P, Wang X, Miller LD, et al. Hepcidin regulation in prostate and its disruption in prostate cancer. Cancer Res. 2015;75(11):2254-63.
306. Evans P, Cindrova-Davies T, Muttukrishna S, Burton GJ, Porter J, Jauniaux E. Hepcidin and iron species distribution inside the first-trimester human gestational sac. Mol Hum Reprod. 2011;17(4):227-32.
307. Gnana-Prakasam JP, Martin PM, Mysona BA, Roon P, Smith SB, Ganapathy V. Hepcidin expression in mouse retina and its regulation via lipopolysaccharide/Toll-like receptor-4 pathway independent of Hfe. Biochem J. 2008;411(1):79-88.
308. Camaschella C, Nai A, Silvestri L. Iron metabolism and iron disorders revisited in the hepcidin era. Haematologica. 2020;105(2):260-72.
309. Scaramellini N, Fischer D, Agarvas AR, Motta I, Muckenthaler MU, Mertens C. Interpreting Iron Homeostasis in Congenital and Acquired Disorders. Pharmaceuticals (Basel). 2023;16(3).
310. Babitt JL, Huang FW, Wrighting DM, Xia Y, Sidis Y, Samad TA, et al. Bone morphogenetic protein signaling by hemojuvelin regulates hepcidin expression. Nat Genet. 2006;38(5):531-9.
311. Silvestri L, Nai A, Dulja A, Pagani A. Hepcidin and the BMP-SMAD pathway: An unexpected liaison. Vitam Horm. 2019;110:71-99.
312. Meynard D, Kautz L, Darnaud V, Canonne-Hergaux F, Coppin H, Roth MP. Lack of the bone morphogenetic protein BMP6 induces massive iron overload. Nat Genet. 2009;41(4):478-81.
313. Koch PS, Olsavszky V, Ulbrich F, Sticht C, Demory A, Leibing T, et al. Angiocrine Bmp2 signaling in murine liver controls normal iron homeostasis. Blood. 2017;129(4):415-9.
314. Little SC, Mullins MC. Bone morphogenetic protein heterodimers assemble heteromeric type I receptor complexes to pattern the dorsoventral axis. Nat Cell Biol. 2009;11(5):637-43.
315. Nohno T, Ishikawa T, Saito T, Hosokawa K, Noji S, Wolsing DH, et al. Identification of a human type II receptor for bone morphogenetic protein-4 that forms differential heteromeric complexes with bone morphogenetic protein type I receptors. J Biol Chem. 1995;270(38):22522-6.
316. Huang FW, Pinkus JL, Pinkus GS, Fleming MD, Andrews NC. A mouse model of juvenile hemochromatosis. J Clin Invest. 2005;115(8):2187-91.
317. Casanovas G, Mleczko-Sanecka K, Altamura S, Hentze MW, Muckenthaler MU. Bone morphogenetic protein (BMP)-responsive elements located in the proximal and distal hepcidin promoter are critical for its response to HJV/BMP/SMAD. J Mol Med (Berl). 2009;87(5):471-80.
318. Canali S, Wang CY, Zumbrennen-Bullough KB, Bayer A, Babitt JL. Bone morphogenetic protein 2 controls iron homeostasis in mice independent of Bmp6. Am J Hematol. 2017;92(11):1204-13.
319. Kautz L, Jung G, Valore EV, Rivella S, Nemeth E, Ganz T. Author Correction: Identification of erythroferrone as an erythroid regulator of iron metabolism. Nat Genet. 2020;52(4):463.
320. Lim PJ, Duarte TL, Arezes J, Garcia-Santos D, Hamdi A, Pasricha SR, et al. Nrf2 controls iron homeostasis in haemochromatosis and thalassaemia via Bmp6 and hepcidin. Nat Metab. 2019;1(5):519-31.
321. Csiszar A, Ahmad M, Smith KE, Labinskyy N, Gao Q, Kaley G, et al. Bone morphogenetic protein-2 induces proinflammatory endothelial phenotype. Am J Pathol. 2006;168(2):629-38.
322. Daher R, Kannengiesser C, Houamel D, Lefebvre T, Bardou-Jacquet E, Ducrot N, et al. Heterozygous Mutations in BMP6 Pro-peptide Lead to Inappropriate Hepcidin Synthesis and Moderate Iron Overload in Humans. Gastroenterology. 2016;150(3):672-83.e4.
323. Papanikolaou G, Samuels ME, Ludwig EH, MacDonald ML, Franchini PL, Dubé MP, et al. Mutations in HFE2 cause iron overload in chromosome 1q-linked juvenile hemochromatosis. Nat Genet. 2004;36(1):77-82.

324. Lin L, Nemeth E, Goodnough JB, Thapa DR, Gabayan V, Ganz T. Soluble hemojuvelin is released by proprotein convertase-mediated cleavage at a conserved polybasic RNRR site. Blood Cells Mol Dis. 2008;40(1):122-31.
325. Hanyu A, Ishidou Y, Ebisawa T, Shimanuki T, Imamura T, Miyazono K. The N domain of Smad7 is essential for specific inhibition of transforming growth factor-beta signaling. J Cell Biol. 2001;155(6):1017-27.
326. Du X, She E, Gelbart T, Truksa J, Lee P, Xia Y, et al. The serine protease TMPRSS6 is required to sense iron deficiency. Science. 2008;320(5879):1088-92.
327. Silvestri L, Pagani A, Nai A, De Domenico I, Kaplan J, Camaschella C. The serine protease matriptase-2 (TMPRSS6) inhibits hepcidin activation by cleaving membrane hemojuvelin. Cell Metab. 2008;8(6):502-11.
328. Krijt J, Fujikura Y, Ramsay AJ, Velasco G, Nečas E. Liver hemojuvelin protein levels in mice deficient in matriptase-2 (Tmprss6). Blood Cells Mol Dis. 2011;47(2):133-7.
329. Wahedi M, Wortham AM, Kleven MD, Zhao N, Jue S, Enns CA, et al. Matriptase-2 suppresses hepcidin expression by cleaving multiple components of the hepcidin induction pathway. J Biol Chem. 2017;292(44):18354-71.
330. Krijt J, Frýdlová J, Gurieva I, Přikryl P, Báječný M, Steinbicker AU, et al. Matriptase-2 and Hemojuvelin in Hepcidin Regulation: In Vivo Immunoblot Studies in. Int J Mol Sci. 2021;22(5).
331. Finberg KE, Heeney MM, Campagna DR, Aydinok Y, Pearson HA, Hartman KR, et al. Mutations in TMPRSS6 cause iron-refractory iron deficiency anemia (IRIDA). Nat Genet. 2008;40(5):569-71.
332. Heeney MM, Finberg KE. Iron-refractory iron deficiency anemia (IRIDA). Hematol Oncol Clin North Am. 2014;28(4):637-52, v.
333. De Falco L, Sanchez M, Silvestri L, Kannengiesser C, Muckenthaler MU, Iolascon A, et al. Iron refractory iron deficiency anemia. Haematologica. 2013;98(6):845-53.
334. Meynard D, Vaja V, Sun CC, Corradini E, Chen S, López-Otín C, et al. Regulation of TMPRSS6 by BMP6 and iron in human cells and mice. Blood. 2011;118(3):747-56.
335. Maurer E, Gütschow M, Stirnberg M. Matriptase-2 (TMPRSS6) is directly up-regulated by hypoxia inducible factor-1: identification of a hypoxia-responsive element in the TMPRSS6 promoter region. Biol Chem. 2012;393(6):535-40.
336. Lee P, Hsu MH, Welser-Alves J, Peng H. Severe microcytic anemia but increased erythropoiesis in mice lacking Hfe or Tfr2 and Tmprss6. Blood Cells Mol Dis. 2012;48(3):173-8.
337. Merryweather-Clarke AT, Pointon JJ, Jouanolle AM, Rochette J, Robson KJ. Geography of HFE C282Y and H63D mutations. Genet Test. 2000;4(2):183-98.
338. Nai A, Lidonnici MR, Rausa M, Mandelli G, Pagani A, Silvestri L, et al. The second transferrin receptor regulates red blood cell production in mice. Blood. 2015;125(7):1170-9.
339. Gao J, Chen J, Kramer M, Tsukamoto H, Zhang AS, Enns CA. Interaction of the hereditary hemochromatosis protein HFE with transferrin receptor 2 is required for transferrin-induced hepcidin expression. Cell Metab. 2009;9(3):217-27.
340. Corradini E, Rozier M, Meynard D, Odhiambo A, Lin HY, Feng Q, et al. Iron regulation of hepcidin despite attenuated Smad1,5,8 signaling in mice without transferrin receptor 2 or Hfe. Gastroenterology. 2011;141(5):1907-14.
341. Zheng H, Yang F, Deng K, Wei J, Liu Z, Zheng YC, et al. Relationship between iron overload caused by abnormal hepcidin expression and liver disease: A review. Medicine (Baltimore). 2023;102(11):e33225.
342. Wang CY, Babitt JL. Hepcidin regulation in the anemia of inflammation. Curr Opin Hematol. 2016;23(3):189-97.
343. Ganz T, Nemeth E. Iron Balance and the Role of Hepcidin in Chronic Kidney Disease. Semin Nephrol. 2016;36(2):87-93.
344. Arezes J, Jung G, Gabayan V, Valore E, Ruchala P, Gulig PA, et al. Hepcidin-induced hypoferremia is a critical host defense mechanism against the siderophilic bacterium Vibrio vulnificus. Cell Host Microbe. 2015;17(1):47-57.

345. Leal SM, Roy S, Vareechon C, Carrion S, Clark H, Lopez-Berges MS, et al. Targeting iron acquisition blocks infection with the fungal pathogens Aspergillus fumigatus and Fusarium oxysporum. PLoS Pathog. 2013;9(7):e1003436.
346. Barber MF, Elde NC. Escape from bacterial iron piracy through rapid evolution of transferrin. Science. 2014;346(6215):1362-6.
347. Stefanova D, Raychev A, Deville J, Humphries R, Campeau S, Ruchala P, et al. Hepcidin Protects against Lethal Escherichia coli Sepsis in Mice Inoculated with Isolates from Septic Patients. Infect Immun. 2018;86(7).
348. Pietrangelo A. Genetics, Genetic Testing, and Management of Hemochromatosis: 15 Years Since Hepcidin. Gastroenterology. 2015;149(5):1240-51.e4.
349. Misslinger M, Hortschansky P, Brakhage AA, Haas H. Fungal iron homeostasis with a focus on Aspergillus fumigatus. Biochim Biophys Acta Mol Cell Res. 2021;1868(1):118885.
350. Darton TC, Blohmke CJ, Giannoulatou E, Waddington CS, Jones C, Sturges P, et al. Rapidly Escalating Hepcidin and Associated Serum Iron Starvation Are Features of the Acute Response to Typhoid Infection in Humans. PLoS Negl Trop Dis. 2015;9(9):e0004029.
351. Shike H, Lauth X, Westerman ME, Ostland VE, Carlberg JM, Van Olst JC, et al. Bass hepcidin is a novel antimicrobial peptide induced by bacterial challenge. Eur J Biochem. 2002;269(8):2232-7.
352. Kemna EH, Kartikasari AE, van Tits LJ, Pickkers P, Tjalsma H, Swinkels DW. Regulation of hepcidin: insights from biochemical analyses on human serum samples. Blood Cells Mol Dis. 2008;40(3):339-46.
353. Wrighting DM, Andrews NC. Interleukin-6 induces hepcidin expression through STAT3. Blood. 2006;108(9):3204-9.
354. Pietrangelo A, Dierssen U, Valli L, Garuti C, Rump A, Corradini E, et al. STAT3 is required for IL-6-gp130-dependent activation of hepcidin in vivo. Gastroenterology. 2007;132(1):294-300.
355. Wang RH, Li C, Xu X, Zheng Y, Xiao C, Zerfas P, et al. A role of SMAD4 in iron metabolism through the positive regulation of hepcidin expression. Cell Metab. 2005;2(6):399-409.
356. Mayeur C, Leyton PA, Kolodziej SA, Yu B, Bloch KD. BMP type II receptors have redundant roles in the regulation of hepatic hepcidin gene expression and iron metabolism. Blood. 2014;124(13):2116-23.
357. Mileson BE, Schwartz RD. The use of locomotor activity as a behavioral screen for neuronal damage following transient forebrain ischemia in gerbils. Neurosci Lett. 1991;128(1):71-6.
358. Verga Falzacappa MV, Casanovas G, Hentze MW, Muckenthaler MU. A bone morphogenetic protein (BMP)-responsive element in the hepcidin promoter controls HFE2-mediated hepatic hepcidin expression and its response to IL-6 in cultured cells. J Mol Med (Berl). 2008;86(5):531-40.
359. Canali S, Core AB, Zumbrennen-Bullough KB, Merkulova M, Wang CY, Schneyer AL, et al. Activin B Induces Noncanonical SMAD1/5/8 Signaling via BMP Type I Receptors in Hepatocytes: Evidence for a Role in Hepcidin Induction by Inflammation in Male Mice. Endocrinology. 2016;157(3):1146-62.
360. Guida C, Altamura S, Klein FA, Galy B, Boutros M, Ulmer AJ, et al. A novel inflammatory pathway mediating rapid hepcidin-independent hypoferremia. Blood. 2015;125(14):2265-75.
361. Agoro R, Mura C. Inflammation-induced up-regulation of hepcidin and down-regulation of ferroportin transcription are dependent on macrophage polarization. Blood Cells Mol Dis. 2016;61:16-25.
362. Meynard D, Sun CC, Wu Q, Chen W, Chen S, Nelson CN, et al. Inflammation regulates TMPRSS6 expression via STAT5. PLoS One. 2013;8(12):e82127.
363. Liu Q, Davidoff O, Niss K, Haase VH. Hypoxia-inducible factor regulates hepcidin via erythropoietin-induced erythropoiesis. J Clin Invest. 2012;122(12):4635-44.
364. Nicolas G, Chauvet C, Viatte L, Danan JL, Bigard X, Devaux I, et al. The gene encoding the iron regulatory peptide hepcidin is regulated by anemia, hypoxia, and inflammation. J Clin Invest. 2002;110(7):1037-44.
365. Ashby DR, Gale DP, Busbridge M, Murphy KG, Duncan ND, Cairns TD, et al. Plasma hepcidin levels are elevated but responsive to erythropoietin therapy in renal disease. Kidney Int. 2009;75(9):976-81.

366. Robach P, Recalcati S, Girelli D, Gelfi C, Aachmann-Andersen NJ, Thomsen JJ, et al. Alterations of systemic and muscle iron metabolism in human subjects treated with low-dose recombinant erythropoietin. Blood. 2009;113(26):6707-15.
367. Ashby DR, Gale DP, Busbridge M, Murphy KG, Duncan ND, Cairns TD, et al. Erythropoietin administration in humans causes a marked and prolonged reduction in circulating hepcidin. Haematologica. 2010;95(3):505-8.
368. Lainé F, Laviolle B, Ropert M, Bouguen G, Morcet J, Hamon C, et al. Early effects of erythropoietin on serum hepcidin and serum iron bioavailability in healthy volunteers. Eur J Appl Physiol. 2012;112(4):1391-7.
369. Sasaki Y, Noguchi-Sasaki M, Yasuno H, Yorozu K, Shimonaka Y. Erythropoietin stimulation decreases hepcidin expression through hematopoietic activity on bone marrow cells in mice. Int J Hematol. 2012;96(6):692-700.
370. Tanno T, Bhanu NV, Oneal PA, Goh SH, Staker P, Lee YT, et al. High levels of GDF15 in thalassemia suppress expression of the iron regulatory protein hepcidin. Nat Med. 2007;13(9):1096-101.
371. Casanovas G, Swinkels DW, Altamura S, Schwarz K, Laarakkers CM, Gross HJ, et al. Growth differentiation factor 15 in patients with congenital dyserythropoietic anaemia (CDA) type II. J Mol Med (Berl). 2011;89(8):811-6.
372. Lakhal S, Talbot NP, Crosby A, Stoepker C, Townsend AR, Robbins PA, et al. Regulation of growth differentiation factor 15 expression by intracellular iron. Blood. 2009;113(7):1555-63.
373. Casanovas G, Vujić Spasic M, Casu C, Rivella S, Strelau J, Unsicker K, et al. The murine growth differentiation factor 15 is not essential for systemic iron homeostasis in phlebotomized mice. Haematologica. 2013;98(3):444-7.
374. Hao S, Xiang J, Wu DC, Fraser JW, Ruan B, Cai J, et al. Gdf15 regulates murine stress erythroid progenitor proliferation and the development of the stress erythropoiesis niche. Blood Adv. 2019;3(14):2205-17.
375. Matte A, De Franceschi L. Oxidation and erythropoiesis. Curr Opin Hematol. 2019;26(3):145-51.
376. Tamary H, Shalev H, Perez-Avraham G, Zoldan M, Levi I, Swinkels DW, et al. Elevated growth differentiation factor 15 expression in patients with congenital dyserythropoietic anemia type I. Blood. 2008;112(13):5241-4.
377. Finkenstedt A, Bianchi P, Theurl I, Vogel W, Witcher DR, Wroblewski VJ, et al. Regulation of iron metabolism through GDF15 and hepcidin in pyruvate kinase deficiency. Br J Haematol. 2009;144(5):789-93.
378. Kautz L, Nemeth E. Molecular liaisons between erythropoiesis and iron metabolism. Blood. 2014;124(4):479-82.
379. Pinto JP, Ribeiro S, Pontes H, Thowfeequ S, Tosh D, Carvalho F, et al. Erythropoietin mediates hepcidin expression in hepatocytes through EPOR signaling and regulation of C/EBPalpha. Blood. 2008;111(12):5727-33.
380. Kautz L, Jung G, Valore EV, Rivella S, Nemeth E, Ganz T. Identification of erythroferrone as an erythroid regulator of iron metabolism. Nat Genet. 2014;46(7):678-84.
381. Arezes J, Foy N, McHugh K, Sawant A, Quinkert D, Terraube V, et al. Erythroferrone inhibits the induction of hepcidin by BMP6. Blood. 2018;132(14):1473-7.
382. Wang CY, Xu Y, Traeger L, Dogan DY, Xiao X, Steinbicker AU, et al. Erythroferrone lowers hepcidin by sequestering BMP2/6 heterodimer from binding to the BMP type I receptor ALK3. Blood. 2020;135(6):453-6.
383. Nai A, Rubio A, Campanella A, Gourbeyre O, Artuso I, Bordini J, et al. Limiting hepatic Bmp-Smad signaling by matriptase-2 is required for erythropoietin-mediated hepcidin suppression in mice. Blood. 2016;127(19):2327-36.
384. Aschemeyer S, Gabayan V, Ganz T, Nemeth E, Kautz L. Erythroferrone and matriptase-2 independently regulate hepcidin expression. Am J Hematol. 2017;92(5):E61-E3.

385. Lasocki S, Millot S, Andrieu V, Lettéron P, Pilard N, Muzeau F, et al. Phlebotomies or erythropoietin injections allow mobilization of iron stores in a mouse model mimicking intensive care anemia. Crit Care Med. 2008;36(8):2388-94.
386. Nemeth E. Hepcidin and β-thalassemia major. Blood. 2013;122(1):3-4.
387. Jaspers A, Baron F, Willems E, Seidel L, Wiegerinck ET, Swinkels DW, et al. Serum hepcidin following autologous hematopoietic cell transplantation: an illustration of the interplay of iron status, erythropoiesis and inflammation. Haematologica. 2014;99(3):e35-7.
388. Liu W, Zhang S, Li Q, Wu Y, Jia X, Feng W, et al. Lactate modulates iron metabolism by binding soluble adenylyl cyclase. Cell Metab. 2023;35(9):1597-612.e6.
389. Damian MT, Vulturar R, Login CC, Damian L, Chis A, Bojan A. Anemia in Sports: A Narrative Review. Life (Basel). 2021;11(9).
390. Anand S, Burkenroad A, Glaspy J. Workup of anemia in cancer. Clin Adv Hematol Oncol. 2020;18(10):640-6.
391. Kraut JA, Madias NE. Lactic acidosis. N Engl J Med. 2014;371(24):2309-19.
392. Aregbesola A, Voutilainen S, Virtanen JK, Tuomainen TP. Serum hepcidin concentrations and type 2 diabetes. World J Diabetes. 2015;6(7):978-82.
393. Sardo U, Perrier P, Cormier K, Sotin M, Personnaz J, Medjbeur T, et al. The hepatokine FGL1 regulates hepcidin and iron metabolism during anemia in mice by antagonizing BMP signaling. Blood. 2023.
394. Vecchi C, Montosi G, Zhang K, Lamberti I, Duncan SA, Kaufman RJ, et al. ER stress controls iron metabolism through induction of hepcidin. Science. 2009;325(5942):877-80.
395. Bachman E, Travison TG, Basaria S, Davda MN, Guo W, Li M, et al. Testosterone induces erythrocytosis via increased erythropoietin and suppressed hepcidin: evidence for a new erythropoietin/hemoglobin set point. J Gerontol A Biol Sci Med Sci. 2014;69(6):725-35.
396. Sandnes M, Ulvik RJ, Vorland M, Reikvam H. Hyperferritinemia-A Clinical Overview. J Clin Med. 2021;10(9).
397. Collaborators GA. Prevalence, years lived with disability, and trends in anaemia burden by severity and cause, 1990-2021: findings from the Global Burden of Disease Study 2021. Lancet Haematol. 2023.
398. Guyatt GH, Oxman AD, Ali M, Willan A, McIlroy W, Patterson C. Laboratory diagnosis of iron-deficiency anemia: an overview. J Gen Intern Med. 1992;7(2):145-53.
399. Ganz T, Olbina G, Girelli D, Nemeth E, Westerman M. Immunoassay for human serum hepcidin. Blood. 2008;112(10):4292-7.
400. Denic S, Agarwal MM. Nutritional iron deficiency: an evolutionary perspective. Nutrition. 2007;23(7-8):603-14.
401. de Las Cuevas Allende R, Díaz de Entresotos L, Conde Díez S. Anaemia of chronic diseases: Pathophysiology, diagnosis and treatment. Med Clin (Barc). 2021;156(5):235-42.
402. Bohlius J, Bohlke K, Castelli R, Djulbegovic B, Lustberg MB, Martino M, et al. Management of cancer-associated anemia with erythropoiesis-stimulating agents: ASCO/ASH clinical practice guideline update. Blood Adv. 2019;3(8):1197-210.
403. Adams PC, Reboussin DM, Barton JC, McLaren CE, Eckfeldt JH, McLaren GD, et al. Hemochromatosis and iron-overload screening in a racially diverse population. N Engl J Med. 2005;352(17):1769-78.
404. Adams PC, Barton JC. A diagnostic approach to hyperferritinemia with a non-elevated transferrin saturation. J Hepatol. 2011;55(2):453-8.
405. Brissot P, Ropert M, Le Lan C, Loréal O. Non-transferrin bound iron: a key role in iron overload and iron toxicity. Biochim Biophys Acta. 2012;1820(3):403-10.
406. Brissot P, Pietrangelo A, Adams PC, de Graaff B, McLaren CE, Loréal O. Haemochromatosis. Nat Rev Dis Primers. 2018;4:18016.
407. Allen KJ, Gurrin LC, Constantine CC, Osborne NJ, Delatycki MB, Nicoll AJ, et al. Iron-overload-related disease in HFE hereditary hemochromatosis. N Engl J Med. 2008;358(3):221-30.

408. Golfeyz S, Lewis S, Weisberg IS. Hemochromatosis: pathophysiology, evaluation, and management of hepatic iron overload with a focus on MRI. Expert Rev Gastroenterol Hepatol. 2018;12(8):767-78.
409. França M, Carvalho JG. MR imaging assessment and quantification of liver iron. Abdom Radiol (NY). 2020;45(11):3400-12.
410. Girelli D, Busti F, Brissot P, Cabantchik I, Muckenthaler MU, Porto G. Hemochromatosis classification: update and recommendations by the BIOIRON Society. Blood. 2022;139(20):3018-29.
411. Feder JN, Gnirke A, Thomas W, Tsuchihashi Z, Ruddy DA, Basava A, et al. A novel MHC class I-like gene is mutated in patients with hereditary haemochromatosis. Nat Genet. 1996;13(4):399-408.
412. Merryweather-Clarke AT, Pointon JJ, Shearman JD, Robson KJ, Jouanolle AM, Mosser A, et al. Polymorphism in intron 4 of HFE does not compromise haemochromatosis mutation results. The European Haemochromatosis Consortium. Nat Genet. 1999;23(3):271.
413. Le Gac G, Férec C. The molecular genetics of haemochromatosis. Eur J Hum Genet. 2005;13(11):1172-85.
414. Pietrangelo A. Ferroportin disease: pathogenesis, diagnosis and treatment. Haematologica. 2017;102(12):1972-84.
415. De Domenico I, Ward DM, Musci G, Kaplan J. Iron overload due to mutations in ferroportin. Haematologica. 2006;91(1):92-5.
416. Crosby WH. A history of phlebotomy therapy for hemochromatosis. Am J Med Sci. 1991;301(1):28-31.
417. Shander A, Cappellini MD, Goodnough LT. Iron overload and toxicity: the hidden risk of multiple blood transfusions. Vox Sang. 2009;97(3):185-97.
418. Ganz T, Jung G, Naeim A, Ginzburg Y, Pakbaz Z, Walter PB, et al. Immunoassay for human serum erythroferrone. Blood. 2017;130(10):1243-6.
419. Liu X, Hu J, Hu XR, Li XX, Guan DR, Liu JQ, et al. [Expression of iron-regulating erythroid factors in different types of erythropoiesis disorders]. Zhonghua Xue Ye Xue Za Zhi. 2021;42(1):52-7.
420. Bondu S, Alary AS, Lefèvre C, Houy A, Jung G, Lefebvre T, et al. A variant erythroferrone disrupts iron homeostasis in. Sci Transl Med. 2019;11(500).
421. Rivella S. Iron metabolism under conditions of ineffective erythropoiesis in β-thalassemia. Blood. 2019;133(1):51-8.
422. Camaschella C, Nai A. Ineffective erythropoiesis and regulation of iron status in iron loading anaemias. Br J Haematol. 2016;172(4):512-23.
423. Gattermann N. Pathophysiological and clinical aspects of iron chelation therapy in MDS. Curr Pharm Des. 2012;18(22):3222-34.
424. Athiyarath R, George B, Mathews V, Srivastava A, Edison ES. Association of growth differentiation factor 15 (GDF15) polymorphisms with serum GDF15 and ferritin levels in β-thalassemia. Ann Hematol. 2014;93(12):2093-5.
425. Porter JB, Cappellini MD, Kattamis A, Viprakasit V, Musallam KM, Zhu Z, et al. Iron overload across the spectrum of non-transfusion-dependent thalassaemias: role of erythropoiesis, splenectomy and transfusions. Br J Haematol. 2017;176(2):288-99.
426. Tantawy AA, Adly AA, Ismail EA, Youssef OI, Ali ME. Growth differentiation factor-15 in children and adolescents with thalassemia intermedia: Relation to subclinical atherosclerosis and pulmonary vasculopathy. Blood Cells Mol Dis. 2015;55(2):144-50.
427. Theurl I, Finkenstedt A, Schroll A, Nairz M, Sonnweber T, Bellmann-Weiler R, et al. Growth differentiation factor 15 in anaemia of chronic disease, iron deficiency anaemia and mixed type anaemia. Br J Haematol. 2010;148(3):449-55.
428. de Swart L, Reiniers C, Bagguley T, van Marrewijk C, Bowen D, Hellström-Lindberg E, et al. Labile plasma iron levels predict survival in patients with lower-risk myelodysplastic syndromes. Haematologica. 2018;103(1):69-79.
429. Gattermann N. Iron overload in myelodysplastic syndromes (MDS). Int J Hematol. 2018;107(1):55-63.

430. Jansová H, Šimůnek T. Cardioprotective Potential of Iron Chelators and Prochelators. Curr Med Chem. 2019;26(2):288-301.
431. Hoeks M, Yu G, Langemeijer S, Crouch S, de Swart L, Fenaux P, et al. Impact of treatment with iron chelation therapy in patients with lower-risk myelodysplastic syndromes participating in the European MDS registry. Haematologica. 2020;105(3):640-51.
432. Temraz S, Santini V, Musallam K, Taher A. Iron overload and chelation therapy in myelodysplastic syndromes. Crit Rev Oncol Hematol. 2014;91(1):64-73.
433. Ware RE, de Montalembert M, Tshilolo L, Abboud MR. Sickle cell disease. Lancet. 2017;390(10091):311-23.
434. Grootendorst S, de Wilde J, van Dooijeweert B, van Vuren A, van Solinge W, Schutgens R, et al. The Interplay between Drivers of Erythropoiesis and Iron Homeostasis in Rare Hereditary Anemias: Tipping the Balance. Int J Mol Sci. 2021;22(4).
435. Fertrin KY, Lanaro C, Franco-Penteado CF, de Albuquerque DM, de Mello MR, Pallis FR, et al. Erythropoiesis-driven regulation of hepcidin in human red cell disorders is better reflected through concentrations of soluble transferrin receptor rather than growth differentiation factor 15. Am J Hematol. 2014;89(4):385-90.
436. van Vuren AJ, Eisenga MF, van Straaten S, Glenthøj A, Gaillard CAJM, Bakker SJL, et al. Interplay of erythropoietin, fibroblast growth factor 23, and erythroferrone in patients with hereditary hemolytic anemia. Blood Adv. 2020;4(8):1678-82.
437. Mangaonkar AA, Thawer F, Son J, Ajebo G, Xu H, Barrett NJ, et al. Regulation of iron homeostasis through the erythroferrone-hepcidin axis in sickle cell disease. Br J Haematol. 2020;189(6):1204-9.
438. Hudson KE, Fasano RM, Karafin MS, Hendrickson JE, Francis RO. Mechanisms of alloimmunization in sickle cell disease. Curr Opin Hematol. 2019;26(6):434-41.
439. Mariani R, Trombini P, Pozzi M, Piperno A. Iron metabolism in thalassemia and sickle cell disease. Mediterr J Hematol Infect Dis. 2009;1(1):e2009006.
440. Lee N, Makani J, Tluway F, Makubi A, Armitage AE, Pasricha SR, et al. Decreased Hepcidin Levels Are Associated with Low Steady-state Hemoglobin in Children With Sickle Cell Disease in Tanzania. EBioMedicine. 2018;34:158-64.
441. Karafin MS, Koch KL, Rankin AB, Nischik D, Rahhal G, Simpson P, et al. Erythropoietic drive is the strongest predictor of hepcidin level in adults with sickle cell disease. Blood Cells Mol Dis. 2015;55(4):304-7.
442. Bazinet A, Popradi G. A general practitioner's guide to hematopoietic stem-cell transplantation. Curr Oncol. 2019;26(3):187-91.
443. Appelbaum FR. Hematopoietic-cell transplantation at 50. N Engl J Med. 2007;357(15):1472-5.
444. Passweg JR, Baldomero H, Chabannon C, Basak GW, de la Cámara R, Corbacioglu S, et al. Hematopoietic cell transplantation and cellular therapy survey of the EBMT: monitoring of activities and trends over 30 years. Bone Marrow Transplant. 2021;56(7):1651-64.
445. Niederwieser D, Baldomero H, Bazuaye N, Bupp C, Chaudhri N, Corbacioglu S, et al. One and a half million hematopoietic stem cell transplants: continuous and differential improvement in worldwide access with the use of non-identical family donors. Haematologica. 2022;107(5):1045-53.
446. D'Souza A, Fretham C, Lee SJ, Arora M, Brunner J, Chhabra S, et al. Current Use of and Trends in Hematopoietic Cell Transplantation in the United States. Biol Blood Marrow Transplant. 2020;26(8):e177-e82.
447. Gluckman E, Cappelli B, Bernaudin F, Labopin M, Volt F, Carreras J, et al. Sickle cell disease: an international survey of results of HLA-identical sibling hematopoietic stem cell transplantation. Blood. 2017;129(11):1548-56.
448. Dalle JH, Peffault de Latour R. Allogeneic hematopoietic stem cell transplantation for inherited bone marrow failure syndromes. Int J Hematol. 2016;103(4):373-9.
449. Ljungman P, Bregni M, Brune M, Cornelissen J, de Witte T, Dini G, et al. Allogeneic and autologous transplantation for haematological diseases, solid tumours and immune disorders: current practice in Europe 2009. Bone Marrow Transplant. 2010;45(2):219-34.

450. Klein J, Sato A. The HLA system. First of two parts. N Engl J Med. 2000;343(10):702-9.
451. Klein J, Sato A. The HLA system. Second of two parts. N Engl J Med. 2000;343(11):782-6.
452. Kanakry CG, Fuchs EJ, Luznik L. Modern approaches to HLA-haploidentical blood or marrow transplantation. Nat Rev Clin Oncol. 2016;13(2):132.
453. Giralt S. Reduced-intensity conditioning regimens for hematologic malignancies: what have we learned over the last 10 years? Hematology Am Soc Hematol Educ Program. 2005:384-9.
454. Kottaridis PD, Milligan DW, Chopra R, Chakraverty RK, Chakrabarti S, Robinson S, et al. In vivo CAMPATH-1H prevents graft-versus-host disease following nonmyeloablative stem cell transplantation. Blood. 2000;96(7):2419-25.
455. Slavin S, Nagler A, Naparstek E, Kapelushnik Y, Aker M, Cividalli G, et al. Nonmyeloablative stem cell transplantation and cell therapy as an alternative to conventional bone marrow transplantation with lethal cytoreduction for the treatment of malignant and nonmalignant hematologic diseases. Blood. 1998;91(3):756-63.
456. Baron F, Sandmaier BM. Chimerism and outcomes after allogeneic hematopoietic cell transplantation following nonmyeloablative conditioning. Leukemia. 2006;20(10):1690-700.
457. Tomblyn M, Chiller T, Einsele H, Gress R, Sepkowitz K, Storek J, et al. Guidelines for preventing infectious complications among hematopoietic cell transplantation recipients: a global perspective. Biol Blood Marrow Transplant. 2009;15(10):1143-238.
458. Ruutu T, Eriksson B, Remes K, Juvonen E, Volin L, Remberger M, et al. Ursodeoxycholic acid for the prevention of hepatic complications in allogeneic stem cell transplantation. Blood. 2002;100(6):1977-83.
459. Nash RA, Piñeiro LA, Storb R, Deeg HJ, Fitzsimmons WE, Furlong T, et al. FK506 in combination with methotrexate for the prevention of graft-versus-host disease after marrow transplantation from matched unrelated donors. Blood. 1996;88(9):3634-41.
460. Muffly L, Pasquini MC, Martens M, Brazauskas R, Zhu X, Adekola K, et al. Increasing use of allogeneic hematopoietic cell transplantation in patients aged 70 years and older in the United States. Blood. 2017;130(9):1156-64.
461. Servais S, Beguin Y, Delens L, Ehx G, Fransolet G, Hannon M, et al. Novel approaches for preventing acute graft-versus-host disease after allogeneic hematopoietic stem cell transplantation. Expert Opin Investig Drugs. 2016;25(8):957-72.
462. Cutler CS, Koreth J, Ritz J. Mechanistic approaches for the prevention and treatment of chronic GVHD. Blood. 2017;129(1):22-9.
463. Phelan R, Chen M, Bupp C, Bolon YT, Broglie L, Brunner-Grady J, et al. Updated Trends in Hematopoietic Cell Transplantation in the United States with an Additional Focus on Adolescent and Young Adult Transplantation Activity and Outcomes. Transplant Cell Ther. 2022;28(7):409.e1-.e10.
464. Wong FL, Teh JB, Atencio L, Stiller T, Kim H, Chanson D, et al. Conditional Survival, Cause-Specific Mortality, and Risk Factors of Late Mortality After Allogeneic Hematopoietic Cell Transplantation. J Natl Cancer Inst. 2020;112(11):1153-61.
465. Wingard JR, Majhail NS, Brazauskas R, Wang Z, Sobocinski KA, Jacobsohn D, et al. Long-term survival and late deaths after allogeneic hematopoietic cell transplantation. J Clin Oncol. 2011;29(16):2230-9.
466. Martin PJ, Counts GW, Appelbaum FR, Lee SJ, Sanders JE, Deeg HJ, et al. Life expectancy in patients surviving more than 5 years after hematopoietic cell transplantation. J Clin Oncol. 2010;28(6):1011-6.
467. Bhatia S, Francisco L, Carter A, Sun CL, Baker KS, Gurney JG, et al. Late mortality after allogeneic hematopoietic cell transplantation and functional status of long-term survivors: report from the Bone Marrow Transplant Survivor Study. Blood. 2007;110(10):3784-92.
468. Atsuta Y, Hirakawa A, Nakasone H, Kurosawa S, Oshima K, Sakai R, et al. Late Mortality and Causes of Death among Long-Term Survivors after Allogeneic Stem Cell Transplantation. Biol Blood Marrow Transplant. 2016;22(9):1702-9.

469. Solh MM, Bashey A, Solomon SR, Morris LE, Zhang X, Brown S, et al. Long term survival among patients who are disease free at 1-year post allogeneic hematopoietic cell transplantation: a single center analysis of 389 consecutive patients. Bone Marrow Transplant. 2018;53(5):576-83.
470. Patel SS, Rybicki LA, Corrigan D, Bolwell B, Dean R, Liu H, et al. Prognostic Factors for Mortality among Day +100 Survivors after Allogeneic Hematopoietic Cell Transplantation. Biol Blood Marrow Transplant. 2018;24(5):1029-34.
471. Bejanyan N, Weisdorf DJ, Logan BR, Wang HL, Devine SM, de Lima M, et al. Survival of patients with acute myeloid leukemia relapsing after allogeneic hematopoietic cell transplantation: a center for international blood and marrow transplant research study. Biol Blood Marrow Transplant. 2015;21(3):454-9.
472. Norkin M, Shaw BE, Brazauskas R, Tecca HR, Leather HL, Gea-Banacloche J, et al. Characteristics of Late Fatal Infections after Allogeneic Hematopoietic Cell Transplantation. Biol Blood Marrow Transplant. 2019;25(2):362-8.
473. Armand P, Gibson CJ, Cutler C, Ho VT, Koreth J, Alyea EP, et al. A disease risk index for patients undergoing allogeneic stem cell transplantation. Blood. 2012;120(4):905-13.
474. Armand P, Kim HT, Logan BR, Wang Z, Alyea EP, Kalaycio ME, et al. Validation and refinement of the Disease Risk Index for allogeneic stem cell transplantation. Blood. 2014;123(23):3664-71.
475. Shouval R, Fein JA, Labopin M, Cho C, Bazarbachi A, Baron F, et al. Development and validation of a disease risk stratification system for patients with haematological malignancies: a retrospective cohort study of the European Society for Blood and Marrow Transplantation registry. Lancet Haematol. 2021;8(3):e205-e15.
476. Sorror ML, Maris MB, Storb R, Baron F, Sandmaier BM, Maloney DG, et al. Hematopoietic cell transplantation (HCT)-specific comorbidity index: a new tool for risk assessment before allogeneic HCT. Blood. 2005;106(8):2912-9.
477. Gratwohl A, Stern M, Brand R, Apperley J, Baldomero H, de Witte T, et al. Risk score for outcome after allogeneic hematopoietic stem cell transplantation: a retrospective analysis. Cancer. 2009;115(20):4715-26.
478. Potdar R, Varadi G, Fein J, Labopin M, Nagler A, Shouval R. Prognostic Scoring Systems in Allogeneic Hematopoietic Stem Cell Transplantation: Where Do We Stand? Biol Blood Marrow Transplant. 2017;23(11):1839-46.
479. Parimon T, Au DH, Martin PJ, Chien JW. A risk score for mortality after allogeneic hematopoietic cell transplantation. Ann Intern Med. 2006;144(6):407-14.
480. Socié G, Stone JV, Wingard JR, Weisdorf D, Henslee-Downey PJ, Bredeson C, et al. Long-term survival and late deaths after allogeneic bone marrow transplantation. Late Effects Working Committee of the International Bone Marrow Transplant Registry. N Engl J Med. 1999;341(1):14-21.
481. Deeg HJ, Leisenring W, Storb R, Nims J, Flowers ME, Witherspoon RP, et al. Long-term outcome after marrow transplantation for severe aplastic anemia. Blood. 1998;91(10):3637-45.
482. Duell T, van Lint MT, Ljungman P, Tichelli A, Socié G, Apperley JF, et al. Health and functional status of long-term survivors of bone marrow transplantation. EBMT Working Party on Late Effects and EULEP Study Group on Late Effects. European Group for Blood and Marrow Transplantation. Ann Intern Med. 1997;126(3):184-92.
483. Goldman JM, Majhail NS, Klein JP, Wang Z, Sobocinski KA, Arora M, et al. Relapse and late mortality in 5-year survivors of myeloablative allogeneic hematopoietic cell transplantation for chronic myeloid leukemia in first chronic phase. J Clin Oncol. 2010;28(11):1888-95.
484. Thanarajasingam G, Kim HT, Cutler C, Ho VT, Koreth J, Alyea EP, et al. Outcome and prognostic factors for patients who relapse after allogeneic hematopoietic stem cell transplantation. Biol Blood Marrow Transplant. 2013;19(12):1713-8.
485. Kreidieh F, Abou Dalle I, Moukalled N, El-Cheikh J, Brissot E, Mohty M, et al. Relapse after allogeneic hematopoietic stem cell transplantation in acute myeloid leukemia: an overview of prevention and treatment. Int J Hematol. 2022;116(3):330-40.
486. Lee CJ, Savani BN, Mohty M, Gorin NC, Labopin M, Ruggeri A, et al. Post-remission strategies for the prevention of relapse following allogeneic hematopoietic cell transplantation for high-risk acute

myeloid leukemia: expert review from the Acute Leukemia Working Party of the European Society for Blood and Marrow Transplantation. Bone Marrow Transplant. 2019;54(4):519-30.
487. Coppell JA, Richardson PG, Soiffer R, Martin PL, Kernan NA, Chen A, et al. Hepatic veno-occlusive disease following stem cell transplantation: incidence, clinical course, and outcome. Biol Blood Marrow Transplant. 2010;16(2):157-68.
488. Wah TM, Moss HA, Robertson RJ, Barnard DL. Pulmonary complications following bone marrow transplantation. Br J Radiol. 2003;76(906):373-9.
489. Soubani AO, Miller KB, Hassoun PM. Pulmonary complications of bone marrow transplantation. Chest. 1996;109(4):1066-77.
490. Li Z, Denning DW. The Impact of Corticosteroids on the Outcome of Fungal Disease: a Systematic Review and Meta-analysis. Curr Fungal Infect Rep. 2023;17(1):54-70.
491. Lin SJ, Schranz J, Teutsch SM. Aspergillosis case-fatality rate: systematic review of the literature. Clin Infect Dis. 2001;32(3):358-66.
492. Yang X, Li D, Xie Y. Anti-Thymocyte Globulin Prophylaxis in Patients With Hematological Malignancies Undergoing Allogeneic Hematopoietic Stem Cell Transplantation: An Updated Meta-Analysis. Front Oncol. 2021;11:717678.
493. Finke J, Schmoor C, Bethge WA, Ottinger H, Stelljes M, Volin L, et al. Long-term outcomes after standard graft-versus-host disease prophylaxis with or without anti-human-T-lymphocyte immunoglobulin in haemopoietic cell transplantation from matched unrelated donors: final results of a randomised controlled trial. Lancet Haematol. 2017;4(6):e293-e301.
494. Filipovich AH, Weisdorf D, Pavletic S, Socie G, Wingard JR, Lee SJ, et al. National Institutes of Health consensus development project on criteria for clinical trials in chronic graft-versus-host disease: I. Diagnosis and staging working group report. Biol Blood Marrow Transplant. 2005;11(12):945-56.
495. Jagasia MH, Greinix HT, Arora M, Williams KM, Wolff D, Cowen EW, et al. National Institutes of Health Consensus Development Project on Criteria for Clinical Trials in Chronic Graft-versus-Host Disease: I. The 2014 Diagnosis and Staging Working Group report. Biol Blood Marrow Transplant. 2015;21(3):389-401.e1.
496. Zeiser R, Blazar BR. Acute Graft-versus-Host Disease - Biologic Process, Prevention, and Therapy. N Engl J Med. 2017;377(22):2167-79.
497. Harris AC, Young R, Devine S, Hogan WJ, Ayuk F, Bunworasate U, et al. International, Multicenter Standardization of Acute Graft-versus-Host Disease Clinical Data Collection: A Report from the Mount Sinai Acute GVHD International Consortium. Biol Blood Marrow Transplant. 2016;22(1):4-10.
498. Jagasia M, Arora M, Flowers ME, Chao NJ, McCarthy PL, Cutler CS, et al. Risk factors for acute GVHD and survival after hematopoietic cell transplantation. Blood. 2012;119(1):296-307.
499. Penack O, Marchetti M, Ruutu T, Aljurf M, Bacigalupo A, Bonifazi F, et al. Prophylaxis and management of graft versus host disease after stem-cell transplantation for haematological malignancies: updated consensus recommendations of the European Society for Blood and Marrow Transplantation. Lancet Haematol. 2020;7(2):e157-e67.
500. Zeiser R, von Bubnoff N, Butler J, Mohty M, Niederwieser D, Or R, et al. Ruxolitinib for Glucocorticoid-Refractory Acute Graft-versus-Host Disease. N Engl J Med. 2020;382(19):1800-10.
501. Socié G, Schmoor C, Bethge WA, Ottinger HD, Stelljes M, Zander AR, et al. Chronic graft-versus-host disease: long-term results from a randomized trial on graft-versus-host disease prophylaxis with or without anti-T-cell globulin ATG-Fresenius. Blood. 2011;117(23):6375-82.
502. Sullivan KM, Shulman HM, Storb R, Weiden PL, Witherspoon RP, McDonald GB, et al. Chronic graft-versus-host disease in 52 patients: adverse natural course and successful treatment with combination immunosuppression. Blood. 1981;57(2):267-76.
503. Lee SJ, Vogelsang G, Flowers ME. Chronic graft-versus-host disease. Biol Blood Marrow Transplant. 2003;9(4):215-33.
504. Martin PJ, Rizzo JD, Wingard JR, Ballen K, Curtin PT, Cutler C, et al. First- and second-line systemic treatment of acute graft-versus-host disease: recommendations of the American Society of Blood and Marrow Transplantation. Biol Blood Marrow Transplant. 2012;18(8):1150-63.

505. Zeiser R, Polverelli N, Ram R, Hashmi SK, Chakraverty R, Middeke JM, et al. Ruxolitinib for Glucocorticoid-Refractory Chronic Graft-versus-Host Disease. N Engl J Med. 2021;385(3):228-38.
506. Zeiser R, Burchert A, Lengerke C, Verbeek M, Maas-Bauer K, Metzelder SK, et al. Ruxolitinib in corticosteroid-refractory graft-versus-host disease after allogeneic stem cell transplantation: a multicenter survey. Leukemia. 2015;29(10):2062-8.
507. von Bubnoff N, Ihorst G, Grishina O, Röthling N, Bertz H, Duyster J, et al. Ruxolitinib in GvHD (RIG) study: a multicenter, randomized phase 2 trial to determine the response rate of Ruxolitinib and best available treatment (BAT) versus BAT in steroid-refractory acute graft-versus-host disease (aGvHD) (NCT02396628). BMC Cancer. 2018;18(1):1132.
508. Miller CB, Lazarus HM. Erythropoietin in stem cell transplantation. Bone Marrow Transplant. 2001;27(10):1011-6.
509. Kharfan-Dabaja MA, Kumar A, Ayala E, Aljurf M, Nishihori T, Marsh R, et al. Standardizing Definitions of Hematopoietic Recovery, Graft Rejection, Graft Failure, Poor Graft Function, and Donor Chimerism in Allogeneic Hematopoietic Cell Transplantation: A Report on Behalf of the American Society for Transplantation and Cellular Therapy. Transplant Cell Ther. 2021;27(8):642-9.
510. Baron F, Little MT, Storb R. Kinetics of engraftment following allogeneic hematopoietic cell transplantation with reduced-intensity or nonmyeloablative conditioning. Blood Rev. 2005;19(3):153-64.
511. Zhu X, Tang B, Sun Z. Umbilical cord blood transplantation: Still growing and improving. Stem Cells Transl Med. 2021;10 Suppl 2(Suppl 2):S62-S74.
512. Ballen KK, Gluckman E, Broxmeyer HE. Umbilical cord blood transplantation: the first 25 years and beyond. Blood. 2013;122(4):491-8.
513. Baron F, Vanstraelen G, Beguin Y. Transfusions after nonmyeloablative or reduced-intensity conditioning regimens. Leukemia. 2006;20(12):2081-6.
514. Martino M, Lanza F, Demirer T, Moscato T, Secondino S, Pedrazzoli P, et al. Erythropoiesis-stimulating agents in allogeneic and autologous hematopoietic stem cell transplantation. Expert Opin Biol Ther. 2015;15(2):195-211.
515. Beguin Y, Clemons GK, Oris R, Fillet G. Circulating erythropoietin levels after bone marrow transplantation: inappropriate response to anemia in allogeneic transplants. Blood. 1991;77(4):868-73.
516. Beguin Y, Oris R, Fillet G. Dynamics of erythropoietic recovery following bone marrow transplantation: role of marrow proliferative capacity and erythropoietin production in autologous versus allogeneic transplants. Bone Marrow Transplant. 1993;11(4):285-92.
517. Beguin Y, Baron F, Fillet G. Influence of marrow erythropoietic activity on serum erythropoietin levels after autologous hematopoietic stem cell transplantation. Haematologica. 1998;83(12):1076-81.
518. Ireland RM, Atkinson K, Concannon A, Dodds A, Downs K, Biggs JC. Serum erythropoietin changes in autologous and allogeneic bone marrow transplant patients. Br J Haematol. 1990;76(1):128-34.
519. Schapira L, Antin JH, Ransil BJ, Antman KH, Eder JP, McGarigle CJ, et al. Serum erythropoietin levels in patients receiving intensive chemotherapy and radiotherapy. Blood. 1990;76(11):2354-9.
520. Bowen DT, Janowska-Wieczorek A. Serum erythropoietin following cytostatic therapy. Br J Haematol. 1990;74(3):372-3.
521. Cazzola M, Guarnone R, Cerani P, Centenara E, Rovati A, Beguin Y. Red blood cell precursor mass as an independent determinant of serum erythropoietin level. Blood. 1998;91(6):2139-45.
522. Chapel S, Veng-Pedersen P, Hohl RJ, Schmidt RL, McGuire EM, Widness JA. Changes in erythropoietin pharmacokinetics following busulfan-induced bone marrow ablation in sheep: evidence for bone marrow as a major erythropoietin elimination pathway. J Pharmacol Exp Ther. 2001;298(2):820-4.
523. Lezón C, Alippi RM, Barceló AC, Martínez MP, Conti MI, Bozzini CE. Depression of stimulated erythropoietin production in mice with enhanced erythropoiesis. Haematologica. 1995;80(6):491-4.
524. Miller CB, Jones RJ, Zahurak ML, Piantadosi S, Burns WH, Santos GW, et al. Impaired erythropoietin response to anemia after bone marrow transplantation. Blood. 1992;80(10):2677-82.

525. Bosi A, Vannucchi AM, Grossi A, Guidi S, Saccardi R, Rafanelli D, et al. Inadequate erythropoietin production in allogeneic bone marrow transplant patients. Haematologica. 1991;76(4):280-4.
526. Baron F, Frère P, Fillet G, Beguin Y. Recombinant human erythropoietin therapy is very effective after an autologous peripheral blood stem cell transplant when started soon after engraftment. Clin Cancer Res. 2003;9(15):5566-72.
527. Baron F, Fillet G, Beguin Y. Erythropoiesis after nonmyeloablative stem-cell transplantation is not impaired by inadequate erythropoietin production as observed after conventional allogeneic transplantation. Transplantation. 2002;74(12):1692-6.
528. Abedi MR, Bäckman L, Boström L, Lindbäck B, Ringdén O. Markedly increased serum erythropoietin levels following conditioning for allogeneic bone marrow transplantation. Bone Marrow Transplant. 1990;6(2):121-6.
529. Montanaro D, Gropuzzo M, Tulissi P, Boscutti G, Risaliti A, Baccarani U, et al. Effects of mycophenolate mofetil and azathioprine on the erythropoietin production in renal transplant recipients. Transplant Proc. 2001;33(7-8):3253-5.
530. Ferrara JL, Cooke KR, Teshima T. The pathophysiology of acute graft-versus-host disease. Int J Hematol. 2003;78(3):181-7.
531. Lacey SF, Diamond DJ, Zaia JA. Assessment of cellular immunity to human cytomegalovirus in recipients of allogeneic stem cell transplants. Biol Blood Marrow Transplant. 2004;10(7):433-47.
532. Faquin WC, Schneider TJ, Goldberg MA. Effect of inflammatory cytokines on hypoxia-induced erythropoietin production. Blood. 1992;79(8):1987-94.
533. Holler E, Kolb HJ, Möller A, Kempeni J, Liesenfeld S, Pechumer H, et al. Increased serum levels of tumor necrosis factor alpha precede major complications of bone marrow transplantation. Blood. 1990;75(4):1011-6.
534. Aapro M. An update on twenty years of anemia management with erythropoiesis-stimulating agents in nephrology and oncology/hematology. Oncologist. 2009;14 Suppl 1:1-5.
535. Vannucchi AM, Bosi A, Grossi A, Guidi S, Saccardi R, Bacci P, et al. The use of erythropoietin in the treatment of post-bone marrow transplantation anemia. Int J Artif Organs. 1993;16 Suppl 5:8-12.
536. Locatelli F, Zecca M, Pedrazzoli P, Prete L, Quaglini S, Comoli P, et al. Use of recombinant human erythropoietin after bone marrow transplantation in pediatric patients with acute leukemia: effect on erythroid repopulation in autologous versus allogeneic transplants. Bone Marrow Transplant. 1994;13(4):403-10.
537. Klaesson S, Ringdén O, Ljungman P, Lönnqvist B, Wennberg L. Reduced blood transfusions requirements after allogeneic bone marrow transplantation: results of a randomised, double-blind study with high-dose erythropoietin. Bone Marrow Transplant. 1994;13(4):397-402.
538. Migdady Y, Pang Y, Kalsi SS, Childs R, Arai S. Post-hematopoietic stem cell transplantation immune-mediated anemia: a literature review and novel therapeutics. Blood Adv. 2022;6(8):2707-21.
539. Heyll A, Aul C, Runde V, Arning M, Schneider W, Wernet P. Treatment of pure red cell aplasia after major ABO-incompatible bone marrow transplantation with recombinant erythropoietin. Blood. 1991;77(4):906.
540. Santamaría A, Sureda A, Martino R, Domingo-Albós A, Muñiz-Díaz E, Brunet S. Successful treatment of pure red cell aplasia after major ABO-incompatible T cell-depleted bone marrow transplantation with erythropoietin. Bone Marrow Transplant. 1997;20(12):1105-7.
541. Zhu P, Wu Y, Cui D, Shi J, Yu J, Zhao Y, et al. Prevalence of Pure Red Cell Aplasia Following Major ABO-Incompatible Hematopoietic Stem Cell Transplantation. Front Immunol. 2022;13:829670.
542. Hirokawa M, Fukuda T, Ohashi K, Hidaka M, Ichinohe T, Iwato K, et al. Efficacy and long-term outcome of treatment for pure red cell aplasia after allogeneic stem cell transplantation from major ABO-incompatible donors. Biol Blood Marrow Transplant. 2013;19(7):1026-32.
543. Longval T, Galimard JE, Leprêtre AC, Suarez F, Amiranoff D, Cazaux M, et al. Treatment for pure red cell aplasia after major ABO-incompatible allogeneic stem cell transplantation: a multicentre study. Br J Haematol. 2021;193(4):814-26.

544. Davies SV, Fegan CD, Kendall R, Beguin Y, Cavill I. Serum erythropoietin during autologous bone marrow transplantation: relationship to measures of erythroid activity. Clin Lab Haematol. 1995;17(2):139-44.
545. Baron F, Sautois B, Baudoux E, Matus G, Fillet G, Beguin Y. Optimization of recombinant human erythropoietin therapy after allogeneic hematopoietic stem cell transplantation. Exp Hematol. 2002;30(6):546-54.
546. Baron F, Frère P, Beguin Y. Once weekly recombinant human erythropoietin therapy is very efficient after allogeneic peripheral blood stem cell transplantation when started soon after engraftment. Haematologica. 2003;88(6):718-20.
547. Jaspers A, Baron F, Willems E, Seidel L, Hafraoui K, Vanstraelen G, et al. Erythropoietin therapy after allogeneic hematopoietic cell transplantation: a prospective, randomized trial. Blood. 2014;124(1):33-41.
548. Jaspers A, Baron F, Servais S, Lejeune M, Willems É, Seidel L, et al. Erythropoietin therapy after allogeneic hematopoietic cell transplantation has no impact on long-term survival. Am J Hematol. 2015;90(9):E197-9.
549. Vanstraelen G, Baron F, Willems E, Bonnet C, Hafraoui K, Frère P, et al. Recombinant human erythropoietin therapy after allogeneic hematopoietic cell transplantation with a nonmyeloablative conditioning regimen: low donor chimerism predicts for poor response. Exp Hematol. 2006;34(7):841-50.
550. Beguin Y, Maertens J, De Prijck B, Schots R, Seidel L, Bonnet C, et al. Darbepoetin-alfa and intravenous iron administration after autologous hematopoietic stem cell transplantation: a prospective multicenter randomized trial. Am J Hematol. 2013;88(12):990-6.
551. Jaspers A, Baron F, Maertens J, De Prijck B, Schots R, Bonnet C, et al. Long-term safety follow-up of a randomized trial of darbepoetin alpha and intravenous iron following autologous hematopoietic cell transplantation. Am J Hematol. 2015;90(7):E133-4.
552. Nakamae M, Nakamae H, Koh S, Koh H, Nishimoto M, Nakashima Y, et al. Prognostic value and clinical implication of serum ferritin levels following allogeneic hematopoietic cell transplantation. Acta Haematol. 2015;133(3):310-6.
553. Gordon LI, Brown SG, Tallman MS, Rademaker AW, Weitzman SA, Lazarus HM, et al. Sequential changes in serum iron and ferritin in patients undergoing high-dose chemotherapy and radiation with autologous bone marrow transplantation: possible implications for treatment related toxicity. Free Radic Biol Med. 1995;18(3):383-9.
554. McKay PJ, Murphy JA, Cameron S, Burnett AK, Campbell M, Tansey P, et al. Iron overload and liver dysfunction after allogeneic or autologous bone marrow transplantation. Bone Marrow Transplant. 1996;17(1):63-6.
555. Butt NM, Clark RE. Autografting as a risk factor for persisting iron overload in long-term survivors of acute myeloid leukaemia. Bone Marrow Transplant. 2003;32(9):909-13.
556. Strasser SI, Kowdley KV, Sale GE, McDonald GB. Iron overload in bone marrow transplant recipients. Bone Marrow Transplant. 1998;22(2):167-73.
557. Eisfeld AK, Westerman M, Krahl R, Leiblein S, Liebert UG, Hehme M, et al. Highly Elevated Serum Hepcidin in Patients with Acute Myeloid Leukemia prior to and after Allogeneic Hematopoietic Cell Transplantation: Does This Protect from Excessive Parenchymal Iron Loading? Adv Hematol. 2011;2011:491058.
558. Mahindra A, Bolwell B, Sobecks R, Rybicki L, Pohlman B, Dean R, et al. Elevated pretransplant ferritin is associated with a lower incidence of chronic graft-versus-host disease and inferior survival after myeloablative allogeneic haematopoietic stem cell transplantation. Br J Haematol. 2009;146(3):310-6.
559. Tachibana T, Tanaka M, Takasaki H, Numata A, Maruta A, Ishigatsubo Y, et al. Pre-SCT serum ferritin is a prognostic factor in adult AML, but not ALL. Bone Marrow Transplant. 2011;46(9):1268-9.
560. Akı SZ, Paşaoğlu H, Yeğin ZA, Suyanı E, Demirtaş CY, Ozkurt ZN, et al. Impact of prohepcidin levels and iron parameters on early post-transplantation toxicities. Hematology. 2011;16(5):284-90.

561. Wahlin A, Lorenz F, Fredriksson M, Remberger M, Wahlin BE, Hägglund H. Hyperferritinemia is associated with low incidence of graft versus host disease, high relapse rate, and impaired survival in patients with blood disorders receiving allogeneic hematopoietic stem cell grafts. Med Oncol. 2011;28(2):552-8.
562. Bazuaye GN, Buser A, Gerull S, Tichelli A, Stern M. Prognostic impact of iron parameters in patients undergoing allo-SCT. Bone Marrow Transplant. 2012;47(1):60-4.
563. Tanaka M, Kanamori H, Matsumoto K, Tachibana T, Numata A, Ohashi K, et al. Clinical significance of pretransplant serum ferritin on the outcome of allogeneic hematopoietic SCT: a prospective cohort study by the Kanto Study Group for Cell Therapy. Bone Marrow Transplant. 2015;50(5):727-33.
564. Sucak GT, Yegin ZA, Ozkurt ZN, Aki SZ, Yağci M. Iron overload: predictor of adverse outcome in hematopoietic stem cell transplantation. Transplant Proc. 2010;42(5):1841-8.
565. Fingrut W, Law A, Lam W, Michelis FV, Viswabandya A, Lipton JH, et al. Post-transplant ferritin level predicts outcomes after allogeneic hematopoietic stem cell transplant, independent from pre-transplant ferritin level. Ann Hematol. 2021;100(3):789-98.
566. Brissot E, Savani BN, Mohty M. Management of high ferritin in long-term survivors after hematopoietic stem cell transplantation. Semin Hematol. 2012;49(1):35-42.
567. Konijn AM, Kaplan R, Or R, Matzner Y. Glycosylated serum ferritin in patients with hematological malignancies before and after bone marrow transplantation. Leuk Lymphoma. 1992;7(1-2):151-6.
568. Or R, Matzner Y, Konijn AM. Serum ferritin in patients undergoing bone marrow transplantation. Cancer. 1987;60(5):1127-31.
569. Sahlstedt L, Ebeling F, von Bonsdorff L, Parkkinen J, Ruutu T. Non-transferrin-bound iron during allogeneic stem cell transplantation. Br J Haematol. 2001;113(3):836-8.
570. Pullarkat V. Iron overload in patients undergoing hematopoietic stem cell transplantation. Adv Hematol. 2010;2010.
571. Harrison P, Neilson JR, Marwah SS, Madden L, Bareford D, Milligan DW. Role of non-transferrin bound iron in iron overload and liver dysfunction in long term survivors of acute leukaemia and bone marrow transplantation. J Clin Pathol. 1996;49(10):853-6.
572. Naoum FA, Espósito BP, Ruiz LP, Ruiz MA, Tanaka PY, Sobreira JT, et al. Assessment of labile plasma iron in patients who undergo hematopoietic stem cell transplantation. Acta Haematol. 2014;131(4):222-6.
573. Naoum FA, Espósito BP, Cançado RD. Impact of conditioning and engraftment on iron status in hematopoietic stem cell transplantation: Contribution of labile plasma iron. Hematol Oncol Stem Cell Ther. 2016;9(4):165-7.
574. Dürken M, Nielsen P, Knobel S, Finckh B, Herrnring C, Dresow B, et al. Nontransferrin-bound iron in serum of patients receiving bone marrow transplants. Free Radic Biol Med. 1997;22(7):1159-63.
575. Dürken M, Herrnring C, Finckh B, Nagel S, Nielsen P, Fischer R, et al. Impaired plasma antioxidative defense and increased nontransferrin-bound iron during high-dose chemotherapy and radiochemotherapy preceding bone marrow transplantation. Free Radic Biol Med. 2000;28(6):887-94.
576. Evens AM, Mehta J, Gordon LI. Rust and corrosion in hematopoietic stem cell transplantation: the problem of iron and oxidative stress. Bone Marrow Transplant. 2004;34(7):561-71.
577. Clemens MR, Ladner C, Schmidt H, Ehninger G, Einsele H, Bühler E, et al. Decreased essential antioxidants and increased lipid hydroperoxides following high-dose radiochemotherapy. Free Radic Res Commun. 1989;7(3-6):227-32.
578. Sakamoto S, Kawabata H, Kanda J, Uchiyama T, Mizumoto C, Kitano T, et al. High pretransplant hepcidin levels are associated with poor overall survival and delayed platelet engraftment after allogeneic hematopoietic stem cell transplantation. Cancer Med. 2017;6(1):120-8.
579. Armand P, Kim HT, Rhodes J, Sainvil MM, Cutler C, Ho VT, et al. Iron overload in patients with acute leukemia or MDS undergoing myeloablative stem cell transplantation. Biol Blood Marrow Transplant. 2011;17(6):852-60.

580. Kanda J, Mizumoto C, Kawabata H, Ichinohe T, Tsuchida H, Tomosugi N, et al. Clinical significance of serum hepcidin levels on early infectious complications in allogeneic hematopoietic stem cell transplantation. Biol Blood Marrow Transplant. 2009;15(8):956-62.
581. Altès A, Remacha AF, Sureda A, Martino R, Briones J, Canals C, et al. Iron overload might increase transplant-related mortality in haematopoietic stem cell transplantation. Bone Marrow Transplant. 2002;29(12):987-9.
582. Kataoka K, Nannya Y, Hangaishi A, Imai Y, Chiba S, Takahashi T, et al. Influence of pretransplantation serum ferritin on nonrelapse mortality after myeloablative and nonmyeloablative allogeneic hematopoietic stem cell transplantation. Biol Blood Marrow Transplant. 2009;15(2):195-204.
583. Artz AS, Logan B, Zhu X, Akpek G, Bufarull RM, Gupta V, et al. The prognostic value of serum C-reactive protein, ferritin, and albumin prior to allogeneic transplantation for acute myeloid leukemia and myelodysplastic syndromes. Haematologica. 2016;101(11):1426-33.
584. Chee L, Tacey M, Lim B, Lim A, Szer J, Ritchie D. Pre-transplant ferritin, albumin and haemoglobin are predictive of survival outcome independent of disease risk index following allogeneic stem cell transplantation. Bone Marrow Transplant. 2017;52(6):870-7.
585. Yan Z, Chen X, Wang H, Chen Y, Chen L, Wu P, et al. Effect of pre-transplantation serum ferritin on outcomes in patients undergoing allogeneic hematopoietic stem cell transplantation: A meta-analysis. Medicine (Baltimore). 2018;97(27):e10310.
586. Vaughn JE, Storer BE, Armand P, Raimondi R, Gibson C, Rambaldi A, et al. Design and Validation of an Augmented Hematopoietic Cell Transplantation-Comorbidity Index Comprising Pretransplant Ferritin, Albumin, and Platelet Count for Prediction of Outcomes after Allogeneic Transplantation. Biol Blood Marrow Transplant. 2015;21(8):1418-24.
587. Mahindra A, Bolwell B, Sobecks R, Rybicki L, Pohlman B, Dean R, et al. Elevated ferritin is associated with relapse after autologous hematopoietic stem cell transplantation for lymphoma. Biol Blood Marrow Transplant. 2008;14(11):1239-44.
588. Lim ZY, Fiaccadori V, Gandhi S, Hayden J, Kenyon M, Ireland R, et al. Impact of pre-transplant serum ferritin on outcomes of patients with myelodysplastic syndromes or secondary acute myeloid leukaemia receiving reduced intensity conditioning allogeneic haematopoietic stem cell transplantation. Leuk Res. 2010;34(6):723-7.
589. Tachibana T, Takasaki H, Tanaka M, Maruta A, Hyo R, Ishigatsubo Y, et al. Serum ferritin and disease status at transplantation predict the outcome of allo-SCT in patients with AML or myelodysplastic syndrome. Bone Marrow Transplant. 2011;46(1):150-1.
590. Jang JE, Kim SJ, Cheong JW, Hyun SY, Kim YD, Kim YR, et al. Early CMV replication and subsequent chronic GVHD have a significant anti-leukemic effect after allogeneic HSCT in acute myeloid leukemia. Ann Hematol. 2015;94(2):275-82.
591. Sakamoto S, Kawabata H, Kanda J, Uchiyama T, Mizumoto C, Kondo T, et al. Differing impacts of pretransplant serum ferritin and C-reactive protein levels on the incidence of chronic graft-versus-host disease after allogeneic hematopoietic stem cell transplantation. Int J Hematol. 2013;97(1):109-16.
592. Maradei SC, Maiolino A, de Azevedo AM, Colares M, Bouzas LF, Nucci M. Serum ferritin as risk factor for sinusoidal obstruction syndrome of the liver in patients undergoing hematopoietic stem cell transplantation. Blood. 2009;114(6):1270-5.
593. Kim YR, Kim JS, Cheong JW, Song JW, Min YH. Transfusion-associated iron overload as an adverse risk factor for transplantation outcome in patients undergoing reduced-intensity stem cell transplantation for myeloid malignancies. Acta Haematol. 2008;120(3):182-9.
594. Ozyilmaz E, Aydogdu M, Sucak G, Aki SZ, Ozkurt ZN, Yegin ZA, et al. Risk factors for fungal pulmonary infections in hematopoietic stem cell transplantation recipients: the role of iron overload. Bone Marrow Transplant. 2010;45(10):1528-33.
595. Armand P, Kim HT, Cutler CS, Ho VT, Koreth J, Alyea EP, et al. Prognostic impact of elevated pretransplantation serum ferritin in patients undergoing myeloablative stem cell transplantation. Blood. 2007;109(10):4586-8.

596. Lee JW, Kang HJ, Kim EK, Kim H, Shin HY, Ahn HS. Effect of iron overload and iron-chelating therapy on allogeneic hematopoietic SCT in children. Bone Marrow Transplant. 2009;44(12):793-7.
597. Morado M, Ojeda E, Garcia-Bustos J, Aguado MJ, Arrieta R, Quevedo E, et al. Serum Ferritin as Risk Factor for Veno-occlusive Disease of the Liver. Prospective Cohort Study. Hematology. 1999;4(6):505-12.
598. Ho GT, Parker A, MacKenzie JF, Morris AJ, Stanley AJ. Abnormal liver function tests following bone marrow transplantation: aetiology and role of liver biopsy. Eur J Gastroenterol Hepatol. 2004;16(2):157-62.
599. Hissen AH, Wan AN, Warwas ML, Pinto LJ, Moore MM. The Aspergillus fumigatus siderophore biosynthetic gene sidA, encoding L-ornithine N5-oxygenase, is required for virulence. Infect Immun. 2005;73(9):5493-503.
600. Altes A, Remacha AF, Sarda P, Baiget M, Sureda A, Martino R, et al. Early clinical impact of iron overload in stem cell transplantation. A prospective study. Ann Hematol. 2007;86(6):443-7.
601. Pullarkat V, Blanchard S, Tegtmeier B, Dagis A, Patane K, Ito J, et al. Iron overload adversely affects outcome of allogeneic hematopoietic cell transplantation. Bone Marrow Transplant. 2008;42(12):799-805.
602. Barba P, Valcárcel D, Pérez-Simón JA, Fernández-Avilés F, Piñana JL, Martino R, et al. Impact of hyperferritinemia on the outcome of reduced-intensity conditioning allogeneic hematopoietic cell transplantation for lymphoid malignancies. Biol Blood Marrow Transplant. 2013;19(4):597-601.
603. Kanda J, Mizumoto C, Ichinohe T, Kawabata H, Saito T, Yamashita K, et al. Pretransplant serum ferritin and C-reactive protein as predictive factors for early bacterial infection after allogeneic hematopoietic cell transplantation. Bone Marrow Transplant. 2011;46(2):208-16.
604. Tachibana T, Tanaka M, Takasaki H, Numata A, Ito S, Watanabe R, et al. Pretransplant serum ferritin is associated with bloodstream infections within 100 days of allogeneic stem cell transplantation for myeloid malignancies. Int J Hematol. 2011;93(3):368-74.
605. Sivgin S, Baldane S, Kaynar L, Kurnaz F, Pala C, Ozturk A, et al. Pretransplant serum ferritin level may be a predictive marker for outcomes in patients having undergone allogeneic hematopoietic stem cell transplantation. Neoplasma. 2012;59(2):183-90.
606. Dadwal SS, Tegtmeier B, Liu X, Frankel P, Ito J, Forman SJ, et al. Impact of pretransplant serum ferritin level on risk of invasive mold infection after allogeneic hematopoietic stem cell transplantation. Eur J Haematol. 2015;94(3):235-42.
607. Platzbecker U, Bornhäuser M, Germing U, Stumpf J, Scott BL, Kröger N, et al. Red blood cell transfusion dependence and outcome after allogeneic peripheral blood stem cell transplantation in patients with de novo myelodysplastic syndrome (MDS). Biol Blood Marrow Transplant. 2008;14(11):1217-25.
608. Meyer SC, O'Meara A, Buser AS, Tichelli A, Passweg JR, Stern M. Prognostic impact of posttransplantation iron overload after allogeneic stem cell transplantation. Biol Blood Marrow Transplant. 2013;19(3):440-4.
609. Konuma T, Kato S, Oiwa-Monna M, Tojo A, Takahashi S. Pretransplant hyperferritinemia has no effect on the outcome of myeloablative cord blood transplantation for acute leukemia and myelodysplastic syndrome. Ann Hematol. 2014;93(6):1071-2.
610. Kanda J, Mizumoto C, Kawabata H, Tsuchida H, Tomosugi N, Matsuo K, et al. Serum hepcidin level and erythropoietic activity after hematopoietic stem cell transplantation. Haematologica. 2008;93(10):1550-4.
611. Altes A, Remacha AF, Sarda P, Baiget M, Canals C, Sierra J. The relationship between transferrin saturation and erythropoiesis during stem cell transplantation. Haematologica. 2006;91(7):992-3.
612. Carmine TC, Evans P, Bruchelt G, Evans R, Handgretinger R, Niethammer D, et al. Presence of iron catalytic for free radical reactions in patients undergoing chemotherapy: implications for therapeutic management. Cancer Lett. 1995;94(2):219-26.
613. Atilla E, Toprak SK, Demirer T. Current Review of Iron Overload and Related Complications in Hematopoietic Stem Cell Transplantation. Turk J Haematol. 2017;34(1):1-9.

614. Armand P, Sainvil MM, Kim HT, Rhodes J, Cutler C, Ho VT, et al. Does iron overload really matter in stem cell transplantation? Am J Hematol. 2012;87(6):569-72.
615. Trottier BJ, Burns LJ, DeFor TE, Cooley S, Majhail NS. Association of iron overload with allogeneic hematopoietic cell transplantation outcomes: a prospective cohort study using R2-MRI-measured liver iron content. Blood. 2013;122(9):1678-84.
616. Wermke M, Schmidt A, Middeke JM, Sockel K, von Bonin M, Schönefeldt C, et al. MRI-based liver iron content predicts for nonrelapse mortality in MDS and AML patients undergoing allogeneic stem cell transplantation. Clin Cancer Res. 2012;18(23):6460-8.
617. Virtanen JM, Itälä-Remes MA, Remes KJ, Vahlberg T, Saunavaara JP, Sinisalo M, et al. Prognostic impact of pretransplant iron overload measured with magnetic resonance imaging on severe infections in allogeneic stem cell transplantation. Eur J Haematol. 2013;91(1):85-93.

# II AIMS

In this work, our **first objective** was to investigate erythropoiesis and iron metabolism after allo-HSCT. As described in detail in the introduction, erythropoiesis and iron metabolism are closely linked. The discovery of ERFE has enabled us to understand the inhibitory regulatory mechanism of erythropoiesis on hepcidin. This negative effect of erythropoietic activity on hepcidin production usually, for instance in thalassemia, dominates the positive action of inflammation or IO. Our group has also shown that after auto-HSCT, the main determinants of hepcidin production are iron stores and erythropoietic activity, while inflammation plays a minor role. However, no study has analyzed the link between erythropoiesis and iron parameters by incorporating ERFE and hepcidin assays after allo-HSCT. Erythropoiesis and iron metabolism are profoundly disrupted after allo-HSCT, with insufficient endogenous EPO production (but consequent anemia responding very well to rHuEPO) and very high SF levels. Therefore, in the first part of this work, we sought to extend our knowledge of erythropoiesis and iron metabolism by incorporating ERFE and hepcidin assays (along with other classic iron parameters) in patients who received or not rHuEPO after allo-HSCT. In addition, we followed patients sequentially up to six months posttransplant and performed a detailed study of the determinants of serum hepcidin and ERFE levels at different times.

Our **second objective** was to study the prognostic impact of pretransplant serum ferritin (SF) and serum hepcidin on different outcomes after allo-HSCT. Indeed, several studies have demonstrated the adverse prognostic impact of high pretransplant SF levels on survival and complications after allo-HSCT. Only four studies have investigated the impact of pretransplant hepcidin, with some of them reporting a negative effect on outcomes (one reporting increased bacterial infections and another decreased platelet engraftment and OS, in multivariate analyses). However, these unfavorable associations are the matter of debate since results are contradictory between studies. Moreover, SF has been used as a surrogate marker for IO. However, two studies have observed that IO accurately quantified by MRI prior to transplantation had no impact on survival or complications after allo-HSCT. Some authors have therefore speculated that SF levels are more likely to reflect an inflammatory state linked to the patient's comorbidities or disease. However, no study analyzed the determinants of pretransplant SF and hepcidin neither their impact on outcomes considering a comprehensive array of biological and clinical characteristics. Therefore, our second aim was to analyze the effect of pretransplant SF and serum hepcidin along other iron parameters, biological markers and clinical characteristics (related to patient, disease, donor and transplant procedures) on critical outcomes in a large cohort of 502 patients with at least 5 years of follow-up after allo-HSCT. First, we looked at determinants of pretransplant SF and serum hepcidin levels in this large cohort to re-examine the results of the first part in a much larger group of patients. Second, we examined whether elevated pre-transplant SF or hepcidin levels were independent prognostic markers on survival outcomes (OS, PFS and NRM),

complications (toxicities, infections and GVHD) and hematological recovery (neutrophil, platelet and red blood cell engraftment and transfusions) after allo-HSCT. Finally, we determined which of the two biological markers was most predictive of these outcomes.

# III RESULTS

# 1. Erythroferrone, the key between erythropoiesis and iron metabolism

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RESEARCH ARTICLE

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## Erythroferrone and hepcidin as mediators between erythropoiesis and iron metabolism during allogeneic hematopoietic stem cell transplant

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**Abstract**

Hematopoietic cell transplantation (HCT) brings important alterations in erythropoiesis and iron metabolism. Hepcidin, which regulates iron metabolism, increases in iron overload or inflammation and decreases with iron deficiency or activated erythropoiesis. Erythroferrone (ERFE) is the erythroid regulator of hepcidin. We investigated erythropoiesis and iron metabolism after allogeneic HCT in 70 patients randomized between erythropoietin (EPO) treatment or no EPO, by serially measuring hepcidin, ERFE, CRP (inflammation), soluble transferrin receptor (sTfR, erythropoiesis), serum iron and transferrin saturation (Tsat; iron for erythropoiesis) and ferritin (iron stores). We identified biological and clinical factors associated with serum hepcidin and ERFE levels. Serum ERFE correlated overall with sTfR and reticulocytes and inversely with hepcidin. Erythroferrone paralleled sTfR levels, dropping during conditioning and recovering with engraftment. Inversely, hepcidin peaked after conditioning and decreased during engraftment. Erythroferrone and hepcidin were not significantly different with or without EPO. Multivariate analyses showed that the major determinant of ERFE was erythropoiesis (sTfR, reticulocytes or serum Epo). Pretransplant hepcidin was associated with previous RBC transfusions and ferritin. After transplantation, the major determinants of hepcidin were iron status (ferritin at all time points and Tsat at day 56) and erythropoiesis (sTfR or reticulocytes or ERFE), while the impact of inflammation was less clear and clinical parameters had no detectable influence. Hepcidin remained significantly higher in patients with high compared to low pretransplant ferritin. After allogeneic HCT with or without EPO therapy, significant alterations of hepcidin occur between pretransplant and day 180, in correlation with iron status and inversely with erythroid ERFE.

## 1.1 Introduction

Erythropoiesis and iron metabolism are closely related, as iron is critical to produce RBC, and erythropoiesis is an essential actor in the regulation of hepcidin, the regulator of iron metabolism (1).

Hepcidin limits iron absorption by the gut and iron release by macrophages and hepatocytes through binding to and degradation of ferroportin, the iron exporter protein, or through occlusion of its central cavity (2-5). Hepcidin synthesis is up-regulated by IO and inflammation leading to reduced iron availability, and down-regulated by iron deficiency, anemia, erythropoiesis and hypoxia, thereby better meeting iron requirements during erythrocyte production (3, 5).

Iron regulates hepcidin production through BMP-2 and 6, acting via their receptors and co-receptors including HJV. This induces a phosphorylation cascade leading to activation of the SMAD1/5/8 complex that binds to SMAD4, translocates into the nucleus, binds BMP response elements, and induces HAMP gene transcription (5-7). Matriptase-2, a protease encoded by the TMPRSS6 gene, causes reduced activation of the BMP-SMAD pathway upon BMP binding to its receptor and HJV (5, 8). Mutations in matriptase thus induce large excesses of hepcidin leading to IRIDA (9). Iron also regulates hepcidin expression by activating the SMAD pathway through the HFE/TFR1/TFR2 complex independently of BMP (5, 10).

Inflammation-based regulation is mainly dependent on inflammatory cytokines such as IL-6, which upregulates hepcidin expression by activating the IL-6 receptor-JAK2-STAT3 pathway, the later binding to STAT3-responsive elements in the hepcidin promoter to induce its transcription (5, 11, 12). In addition, the BMP-SMAD pathway is also involved in hepcidin production during inflammation (12).

Erythropoiesis inhibits hepcidin expression more efficiently than anemia or hypoxia (13). A rapid, persistent drop in hepcidin levels was observed after injection of EPO in mice (14), healthy volunteers (15) and patients with renal failure (16). In 2008, Pinto demonstrated the regulation of hepcidin expression by EPO, in a dose-dependent manner, suggesting the contribution of a transcription factor in the response of hepcidin to EPO (17). Later, Kautz identified the erythroid regulator of iron metabolism in ERFE, establishing the link between EPO and hepcidin (18). ERFE is released by erythroblasts through STAT-5 activation upon EPO stimulation and mediates inhibition of hepcidin production when erythropoiesis is stimulated by EPO in mice (18) and humans (19), but matriptase is also essential for regulation of hepcidin by erythropoiesis (8). Animal models of inflammation and thalassemia have demonstrated that this negative erythropoietic regulator dominates the positive effect of inflammation (20) or IO (21) in hepcidin regulation. We also previously showed that, after auto-HSCT, the major determinants of hepcidin production were iron stores and erythropoietic activity while inflammation exerted a minor role (22).

HSCT offers the opportunity to recapitulate the development of erythropoietic activity from newly developed precursors through fully recovered erythropoiesis. We previously demonstrated that HSCT is associated with EPO deficiency (23-29) and that HSCT-associated anemia is exquisitely sensitive to EPO therapy after autologous (23, 30, 31) and allogeneic (31, 32) HSCT. Efficacy of EPO therapy is further enhanced by iron supplementation (30, 33-38), without significant short-term or long-term toxicity (33, 39), as now recommended in published guidelines (40).

Here we aimed to broaden our knowledge of erythropoiesis and iron metabolism during the first 6 months after allogeneic HSCT. We investigated longitudinally serum levels of: CRP (marker of inflammation); soluble transferrin receptor (sTfR, quantitative marker of erythropoietic activity); SeFe; Tsat (markers of iron availability for erythropoiesis) and SF (marker of iron stores); hepcidin (regulator of iron metabolism); and ERFE (erythroid regulator of hepcidin). We compared their evolution with or without ESAs according to two different schedules. We also examined biological and clinical factors correlated with serum hepcidin and ERFE levels measured at different time points during follow-up.

## 1.2 Patients and methods

### 1.2.1 Study population

We previously published (41) the results of a prospective trial in which 119 patients undergoing allo-HSCT with PBSC for malignant or non-malignant diseases at the University Hospital of Liège were randomized between treatment with weekly EPO (Neorecormon®, kindly provided by Roche) (n=57) or no EPO treatment (n=62).

There were 3 cohorts of patients, i.e. those undergoing MA conditioning who started EPO at day 28 posttransplant (control arm=19; EPO arm=15), and those undergoing NMA conditioning with EPO started at either day 1 (control arm=25; EPO arm=23) or day 28 (control arm=18; EPO arm=19). Complete blood counts, Tsat and SF were measured routinely and biobanking serum samples were collected prospectively before conditioning and weekly thereafter through day 180 posttransplant. The trial was approved by the Ethics Committee of the University Hospital of Liège under number 2003/59 and patients (or his/her guardian if of minor age) signed an informed consent for the clinical study, as well as to collect and analyze blood samples. The primary endpoint of achieving a normal Hb value ≥ 13 gr/dL was reached by 63.1 % in the EPO arm after a median time of 90 days vs 8.1 % in the control arm ($p<0.0001$).

Among these 119 patients, we excluded those who experienced death, relapse, or graft rejection and those given donor lymphocyte infusion the first 100 days after transplantation. Patients without sufficient stored samples were also excluded. Consequently, 70 patients were included in the current study, 35 patients in the EPO arm and 35 in the control arm. There were 24 patients in cohort 1, 25 in cohort 2 and 21 in cohort 3. In the EPO arm, 4 patients received 300 mg of IV

iron saccharate (Venofer®) for functional iron deficiency (Tsat < 20 %): 1 patient in cohort 1 received IV iron at day 120, 1 in cohort 2 at day 87, and 2 in cohort 3 at day 120 or 130, respectively. Iron was omitted in patients with severe IO (SF >2,500 ng/mL without inflammation or liver damage). Control patients never received IV iron. Clinical and biological characteristics of patients are described in **Table 1** and **Table 2**, respectively. Posttransplant complications, including infections and acute or chronic GVHD, were recorded.

### 1.2.2 Laboratory measurements

Laboratory data were monitored from prior to administration of the conditioning regimen (day -10) until day 180 posttransplant, partly on fresh blood and partly on serum samples stored at −80°C until analysis.

Complete blood counts (Hb, white blood cells (WBC) and differential, platelets, percentage, and absolute reticulocyte counts) and percentage of hypochromic red cells (38, 44, 45), CRP, creatinine, liver function tests (bilirubin, aspartame amino transferase (ASAT), lactate dehydrogenase (LDH), albumin and uric acid. Parameters of iron metabolism (SeFe, Tsat and SF were measured weekly using standard laboratory techniques. The SF peak was defined as the highest value between day 0 and day 100 posttransplant.

sTfR, a quantitative measure of total erythropoietic activity, was measured weekly on stored samples by a commercially available ELISA (R&D, Minneapolis, MN), with normal values ranging from 3,000 to 7,000 µg/L, as previously reported (44, 46-49).

Serum hepcidin-25 was quantified on serum samples stored before conditioning and on days 0, 7, 14, 28, 60, 100 and 180, by a liquid chromatography coupled to triple quadrupole mass spectrometry method validated, among others, by the MS-8 lab of our institution (50).

Serum ERFE was measured on stored samples at the same time points by a specific ELISA kit (Intrinsic Lifesciences, The Bio Iron Company, USA, SKU# ERF-001) in 62 patients (31 controls and 31 in EPO arm). The kit has a detection range of 0.16-10 ng/ml.

Serum EPO levels were measured by a commercially available radioimmunoassay (Incstar Corp., Stillwater, MN), as previously published (51, 52), before starting EPO administration, i.e., at day 28 posttransplant.

### 1.2.3 Statistical analyses

Results were expressed as mean ± standard deviation (SD) unless otherwise stated. Some parameters underwent a logarithm or a square root transformation to normalize their distribution (logarithm for SF, SeFe, Tsat, sTfR, CRP, creatinine, bilirubin, ASAT, LDH, Hepcidin, EPO and SF peak; square root for WBC and differential, platelets, and reticulocytes).

Student's t tests were used to compare the means of biological parameters between the control and EPO arms at each predetermined time over a follow-up of 6 months in the 3 cohorts and in the cumulative group. A generalized linear mixed model (GLMM) was used to compare their evolution with respect to time and treatment arm.

Pearson R correlations were used to assess the relations between biological parameters.

Univariate and multivariate linear regression models were used to explain hepcidin and ERFE at each time point with respect to covariates. All variables whose p-value was < 0.15 in univariate analysis were included in multivariate analysis using the stepwise method. P-values < 0.05 were considered as statistically significant. Clinical parameters (age, sex, diagnosis, DRI, HCT-CI score, performance status (PS), conditioning type, donor type, transplant source, ABO compatibility, pretransplant RBC and platelet transfusions, CMV status, GVHD prophylaxis, infections, and EPO treatment) were tested in univariate analyses but did not qualify for multivariate analyses.

In multivariate analyses, we reported r-square value ($R^2$) to give a global appreciation of the accuracy of the model, as well as beta-coefficients for significant individual parameters.

Statistical analyses were performed in SAS version 9.4 (SAS Institute, Cary, NC, USA) and graphs were done using R version 3.6.1. or GraphPad Prism version 7 (GraphPad Software, San Diego, CA).

**Table 1: Patient clinical characteristics.**

| Clinical characteristics | | All (n=70) | Control arm (n=35) | EPO arm (n=35) | p-value |
|---|---|---|---|---|---|
| **Age (years)** | M ± SD | 49 ± 13 | 48 ± 13 | 51 ± 13 | NS |
| **Sex** | Male | 44 (63%) | 23 (66%) | 21 (60%) | NS |
| | Female | 26 (37%) | 12 (34%) | 14 (40%) | NS |
| **Performance status (PS)** | 0 | 11 (16%) | 6 (17%) | 5 (14%) | NS |
| | 1 | 54 (77%) | 26 (74%) | 28 (80%) | NS |
| | 2 | 5 (7%) | 3 (9%) | 2 (6%) | NS |
| **Disease** | Acute leukemia | 20 (29%) | 9 (26%) | 11 (31%) | NS |
| | MDS or MPN | 12 (17%) | 9 (26%) | 3 (9%) | NS |
| | Lymphoid malignancy | 36 (51%) | 16 (45%) | 20 (57%) | NS |
| | Other | 2 (3%) | 1 (3%) | 1 (3%) | NS |
| **Disease Risk Index (42)** | Low | 17 (24%) | 7 (20%) | 10 (29%) | NS |
| | Intermediate | 34 (49%) | 17 (48%) | 17 (48%) | NS |
| | High | 17 (24%) | 9 (26%) | 8 (23%) | NS |
| | Very high | 2 (3%) | 2 (6%) | 0 | NS |
| **Number treatment lines** | 0 | 7 (10%) | 5 (14%) | 2 (6%) | NS |
| | 1 | 22 (31%) | 8 (23%) | 14 (40%) | NS |
| | 2 or 3 | 30 (43%) | 16 (46%) | 14 (40%) | NS |
| | ≥4 | 11 (16%) | 6 (17%) | 5 (14%) | NS |
| **Pretransplant RBC transfusions M ± SD** | | 17 ± 21 | 14 ± 17 | 21 ± 24 | NS |
| **Pretransplant platelet transfusions M ± SD** | | 14 ± 18 | 10 ± 13 | 17 ± 22 | NS |
| **Comorbidity score (HCT-CI) (43)** | 0 | 48 (69%) | 24 (69%) | 24 (69%) | NS |
| | 1-2 | 13 (18%) | 7 (20%) | 6 (17%) | NS |
| | ≥3 | 9 (13%) | 4 (11%) | 5 (14%) | NS |
| **Transplant number** | First transplant | 43 (61%) | 19 (54%) | 24 (69%) | NS |
| | 2nd or 3rd transplant | 27 (39%) | 16 (46%) | 11 (31%) | NS |
| **CD34+ cell dose ($10^6$/Kg)** (all PBSC) **M ± SD** | | 4.60 ± 2.77 | 4.61 ± 2.85 | 4.58 ± 2.73 | NS |
| **Conditioning** | Myeloablative (MA) | 24 (34%) | 14 (40%) | 10 (29%) | NS |
| | Nonmyeloablative (NMA) | 46 (66%) | 21 (60%) | 25 (71%) | NS |
| **Cohort** | MA, EPO at day 28 | 24 (34%) | 14 (40%) | 10 (29%) | NS |
| | NMA, EPO at day 0 | 25 (36%) | 11 (31%) | 14 (40%) | NS |
| | NMA, EPO at day 28 | 21 (30%) | 10 (29%) | 11 (31%) | NS |
| **ATG** | No | 65 (93%) | 32 (91%) | 33 (94%) | NS |
| | Yes | 5 (7%) | 3 (9%) | 2 (6%) | NS |
| **GVHD prophylaxis** | Tacrolimus-based | 17 (24%) | 8 (23%) | 9 (26%) | NS |
| | Ciclosporin-based | 46 (66%) | 21 (60%) | 25 (71%) | NS |
| | Methotrexate +/- other IS | 7 (10%) | 6 (17%) | 1 (3%) | NS |
| **Donor relationship and HLA** | Related 10/10 | 25 (36%) | 14 (40%) | 11 (31%) | NS |
| | Related HLA-mismatched | 2 (3%) | 2 (6%) | 0 | NS |
| | Unrelated 10/10 | 25 (36%) | 12 (34%) | 13 (38%) | NS |
| | Unrelated HLA-mismatched | 18 (25%) | 7 (20%) | 11 (31%) | NS |
| **ABO compatibility** | Identical | 33 (47%) | 12 (34%) | 21 (60%) | NS |
| | Major mismatch | 11 (16%) | 3 (9%) | 8 (23%) | NS |
| | Minor mismatch | 7 (10%) | 3 (9%) | 4 (11%) | NS |
| | Major + minor mismatch | 19 (27%) | 17 (48%) | 2 (6%) | NS |
| **Donor sex match** | F donor > M recipient | 15 (21%) | 6 (17%) | 9 (26%) | NS |
| | Other | 55 (79%) | 29 (83%) | 26 (74%) | NS |
| **Acute GVHD** | No | 27 (39%) | 11 (31%) | 16 (46%) | NS |
| | Yes | 43 (61%) | 24 (69%) | 19 (54%) | NS |
| **Chronic GVHD** | No | 8 (11%) | 5 (14%) | 3 (9%) | NS |
| | Yes | 62 (89%) | 30 (86%) | 32 (91%) | NS |
| **Infection before day 180** | No | 6 (9%) | 2 (6%) | 2 (6%) | NS |
| | Yes | 64 (91%) | 33 (94%) | 33 (94%) | NS |

*M*: mean; *SD*: standard deviation; *NS*: not significant; *MDS*: myelodysplastic syndrome; *MPN*: myeloproliferative neoplasm; *PBSC*: peripheral blood stem cells; *IV*: intravenous; *ATG*: Anti-thymocyte globulin; *GVHD*: graft-versus-host disease; *IS*: Immunosuppressive drug; *HLA*: human leukocyte antigen; *F*: female; *M*: male.

**Table 2: Patient pretransplant (except *) biological characteristics.**

| Pretransplant (except *) values (M±SD) | All (n=70) | Control arm (n=35) | EPO arm (n=35) | P value |
|---|---|---|---|---|
| **Hemoglobin (g/dL)** | 10.8 ± 2.2 | 10.9 ± 2.0 | 10.8 ± 2.3 | NS |
| **Reticulocyte count ($10^3$/µL)** | 75 ± 61 | 77 ± 74 | 73 ± 46 | NS |
| **WBC ($10^3$/µL)** | 4.8 ± 4.0 | 4.7 ± 3.3 | 5.0 ± 4.6 | NS |
| **Neutrophils ($10^3$/µL)** | 2.6 ± 1.7 | 2.6 ± 2.0 | 2.6 ± 1.4 | NS |
| **Lymphocytes ($10^3$/µL)** | 1.4 ± 2.3 | 1.3 ± 1.0 | 1.6 ± 3.1 | NS |
| **Platelets ($10^3$/µL)** | 153 ± 94 | 158 ± 92 | 148 ± 96 | NS |
| **Serum ferritin (ng/dL)** | 1,149 ± 1,443 | 932 ± 1,142 | 1,366 ± 1,681 | NS |
| **Ferritin peak day 0-100 (ng/mL) *** | 4,295 ± 4,230 | 3,429 ± 2,462 | 5,162 ± 5,369 | NS |
| **Serum iron (µmol/mL)** | 22.4 ± 11.6 | 25.3 ± 11.9 | 19.4 ± 10.7 | NS |
| **Tsat (%)** | 0.52 ± 0.28 | 0.57 ± 0.26 | 0.47 ± 0.28 | NS |
| **sTfR (ng/ml)** | 4,521 ± 3,058 | 4,523 ± 3,383 | 4,519 ± 2,746 | NS |
| **Serum Hepcidin (ng/mL)** | 24.2 ± 20.4 | 20.5 ± 13.8 | 28.0 ± 25.1 | NS |
| **Serum ERFE (ng/mL)** | 3.57 ± 9.88 | 4.09 ± 12.02 | 3.06 ± 7.30 | NS |
| **CRP (mg/dL)** | 10.2 ± 16.8 | 11.1 ± 20.5 | 9.3 ± 12.2 | NS |
| **Total bilirubin (mg/dL)** | 0.53 ± 0.32 | 0.62 ± 0.39 | 0.45 ± 0.20 | NS |
| **ASAT (UI/L)** | 26 ± 29 | 31 ± 39 | 21 ± 11 | NS |
| **LDH (UI/L)** | 379 ± 126 | 408 ± 139 | 350 ± 107 | NS |
| **Albumin (g/L)** | 39.4 ± 19.8 | 38.8 ± 19.9 | 40.0 ± 19.0 | NS |
| **Creatinine (mg/dL)** | 0.93 ± 0.36 | 0.91 ± 0.28 | 0.94 ± 0.42 | NS |
| **Serum Epo at day 28 (ng/mL) *** | 60.8 ± 74.6 | 78.1 ± 84.8 | 44.0 ± 60.0 | NS |

M: mean; SD: standard deviation; NS: not significant; WBC: white blood cells; Tsat: transferrin saturation; sTfR: soluble transferrin receptor; ERFE: erythroferrone; CRP: C-reactive protein; ASAT: aspartate aminotransferase; LDH: lactate dehydrogenase; Epo: erythropoietin.

## 1.3 Results

### 1.3.1 Posttransplant course of iron and erythropoietic parameters

First, we analyzed the means of the various parameters over time between the control and EPO arms.

**Figure 1A** represents the evolution of serum ERFE. After an initial rapid decrease during conditioning until day 7, fast recovery occurred in both arms until day 56, after which ERFE stabilized in the control arm while fluctuating with EPO doses in the EPO arm. GLMM confirmed the effect of time on ERFE levels (p=0.017) but not the effect of treatment arm (control Vs EPO, p=0.85) in the whole cohort nor in the different subgroups (MA with EPO at day 28; NMA with EPO at day 0 and NMA with EPO at day 28). The difference between the two arms was maximum on day 56 without being significant (4.53±9.23 ng/ml in controls Vs 7.16±15.29 ng/ml in EPO arm; p=0.09).

**Figure 1B** displays the evolution sTfR, which was quite similar to that of ERFE: a rapid drop after conditioning until day 7, followed by a sharp increase with engraftment in both arms. However, the EPO arm had significantly higher sTfR levels at each time point from day 42 to day 180 posttransplant. GLMM confirmed the effect of posttransplant time (p<0.0001) and treatment arm (p=0.0001) on sTfR values.

**Figure 1C** shows serum hepcidin over time in the control and EPO arms, with the curve following an inverse kinetics to that of ERFE. Before HSCT, hepcidin values were 20.5±13.8 and 27.98±25.06 ng/mL in the control and EPO arms, respectively. Hepcidin peaked 7 days after HSCT, followed by a progressive decrease until day 56 and stabilization thereafter. GLMM confirmed the effect of time (P<0.001) but not treatment arm (p=0.69) on hepcidin levels.

**Figure 1** also describes the posttransplant course of SF (D), transferrin saturation (E), hemoglobin (F), hypochromic red blood cells (G) and CRP (H), while reticulocyte counts (A), albumin (B), LDH (C) and ASAT (D) are displayed in **Figure 2**. SF peaked 28-42 days after HSCT in the 2 arms, before slowly decreasing to values below those measured at baseline in the EPO arm but remaining higher than at baseline in the control arm. Tsat was highest on day 7, then decreased until day 56, with a significantly larger decrease in the EPO arm (P=0.01). The difference between the 2 arms was most pronounced on day 21 (52±25 % in control Vs 35±22 % in EPO arm, p=0.013). GLMM confirmed the effect of time (p<0.0001) and treatment arm (p=0.014) on Tsat values. After their initial drop following conditioning, Hb and percentages of hypochromic red cells increased much more significantly in the EPO arm (effect of time and treatment arm confirmed in GLMM, p<0.0001). CRP peaked during aplasia and changed more randomly thereafter when few patients were infected at the same time point. GLMM demonstrated the effect of time on CRP levels (p<0.0001) but showed no effect of treatment arm (p=0.56).

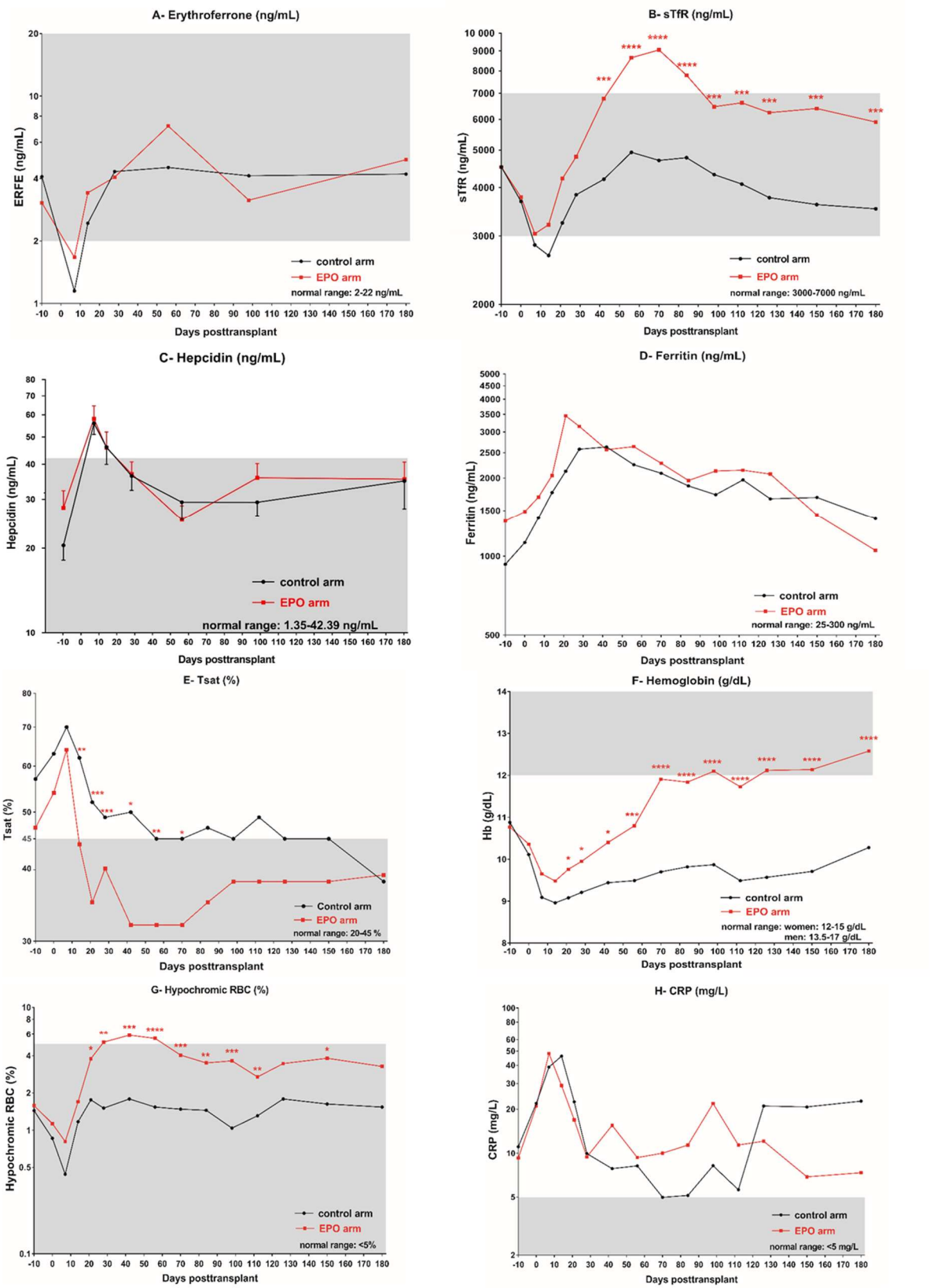


**Figure 1:** Temporal evolution of mean± standard error of the mean (SEM) erythroferrone (A), sTfR (B), hepcidin (C), ferritin (D), transferrin saturation (E), hemoglobin (F), hypochromic red blood cells (G) and CRP (H) following HSCT (day 0), in the **control (in black, n=35)** and **EPO (in red, n=35)** arms. *: <0.05; **: <0.01; ***: <0.001; ****: <0.0001. The gray zone represents the reference interval in normal subjects.

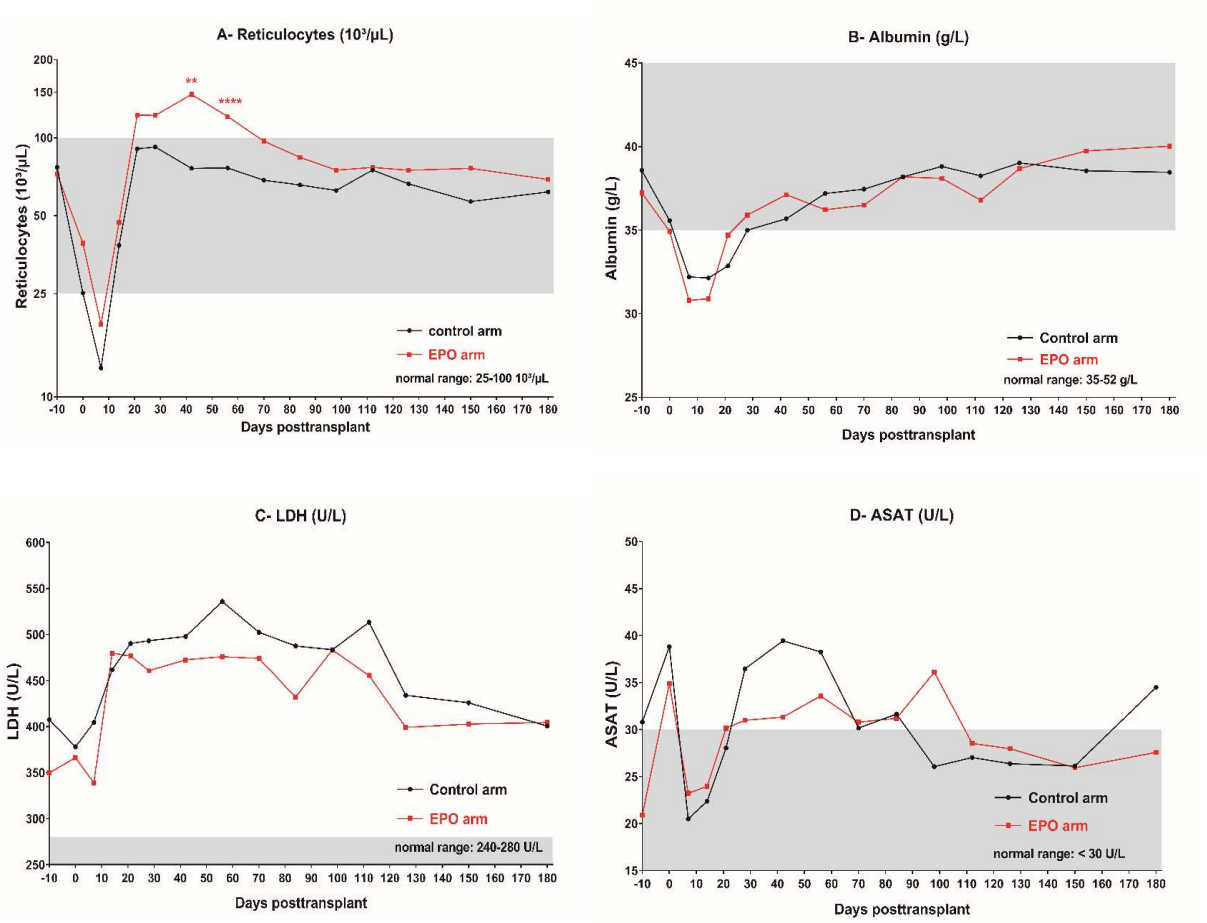


**Figure 2:** Temporal evolution of mean reticulocyte counts (A), serum albumin (B), serum LDH (C) and serum ASAT (D) following HSCT (day 0), in the **control (in black, n=35)** and the **EPO (in red, n=35)** arms. *: < 0.05; **: < 0.01; ***: < 0.001; ****: < 0.0001. The gray zone represents the reference interval in normal subjects.

Serum hepcidin levels have been shown to correlate positively with SF (5) and this was confirmed pretransplant in our study. Therefore, we examined the evolution of serum hepcidin and sTfR (**Figure 3**) in patients separated into two groups based on their pretransplant SF value. The cutoff for SF levels was their median values (≤ or > 820 ng/ml). Baseline hepcidin was significantly higher in patients with higher pretransplant SF (13.9±8.6 vs 4.4±23.6 ng/ml, p<0001) and remained so throughout the posttransplant course (p<0.0001) (**Figure 3A**). When we investigated the course of serum hepcidin in the control and EPO arms separately, there was a small effect of EPO therapy (significant at day 56) in patients with low baseline SF (**Figure 3B**) but not in those with high pretransplant SF levels (**Figure 3C**).

Patients with high baseline SF were more likely to have myeloid than lymphoid malignancies (p=0.0075), had received more RBC (28±25 vs 8±9, p<0.0001) and platelet (20±22 vs 7±9, p<0.001) transfusions and had significantly lower baseline sTfR values (5,193±3,327 vs 3,849±2,644 ng/ml, p=0.014) (**Figure 3D**). During erythropoietic recovery, sTfR increments were

observed in the two arms and were clearly stronger in patients receiving EPO therapy, with no major difference between those with low (**Figure 3E**) or high (**Figure 3F**) baseline SF. Because of a wider range of values, baseline or later ERFE values did not significantly differ between patients with low or high SF.

As type of conditioning probably influences erythropoiesis, we compared the means of the different parameters after MA compared to NMA conditioning. **Figure 4** illustrates the evolution of serum ERFE (A), sTfR (B), hepcidin (C), SF (D), Tsat (E), hemoglobin (F), hypochromic red blood cells (G) and CRP (H) according to conditioning. The overall pattern of evolution remained similar with the two types of conditioning, but the magnitude of change was less prominent after NMA than after MA conditioning, particularly in the early posttransplant period. ERFE at day 7 ($p=0.049$) and sTfR from day 0 until day 14 ($p=0.004$ to $<0.0001$) were significantly lower after MA conditioning, resulting into more anemia ($p=0.007$) until day 112. Hepcidin at days 7 ($p=0.0003$) and 14 ($p=0.04$), SF from day 0 until day 70 ($p=0.02$ to $0.0007$) and Tsat at many time points ($p=0.02$ to $<0.0001$) were higher in patients undergoing MA compared to NMA conditioning. In the NMA cohorts, EPO therapy was started on day 0 in cohort 2 or on day 28 in cohort 3. Changes induced by EPO treatment were quite comparable in their kinetics and range, although a little delayed in patients started on day 28.

Next, we analyzed correlations between ERFE and hepcidin at a given time point and corresponding values at previous and subsequent time, and investigated factors associated with serum hepcidin and ERFE levels overall as well as at each individual time point.

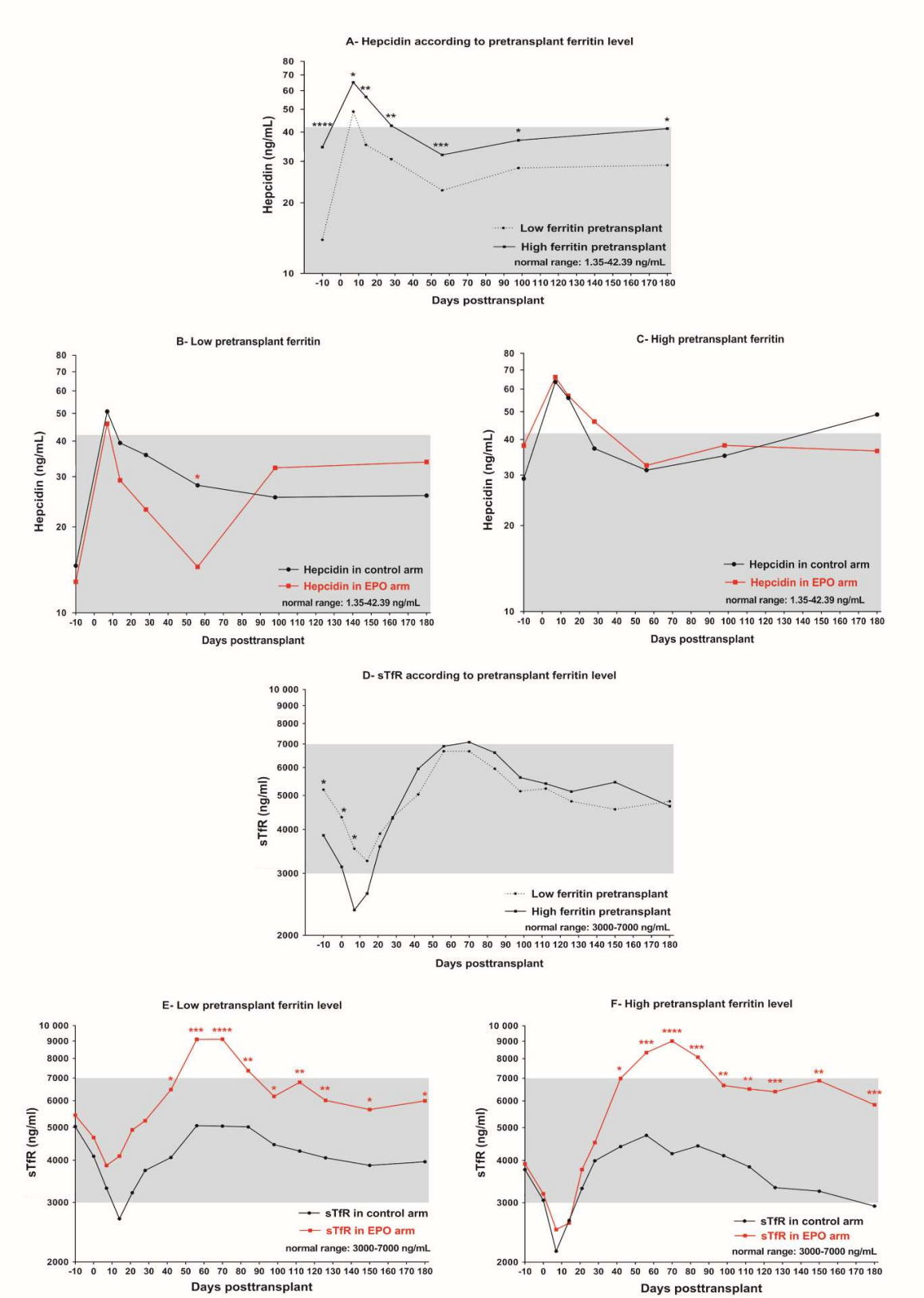


**Figure 3:** Temporal evolution of mean hepcidin (A-B-C) or sTfR (D-E-F) following HSCT (day 0) according to pretransplant ferritin level (the cutoff for pretransplant ferritin level was the median value of 820 ng/ml), in all patients (A, D) as well as in patients with pretransplant ferritin ≤ **(B, E)** or > **(C, F)** the median value of 820 ng/mL in the **control (in black, n=35)** and **EPO (n=35, in red)** arms. *: <0.05; **: <0.01; ***: <0.001; ****: <0.0001. The gray zone represents the reference interval in normal subjects.

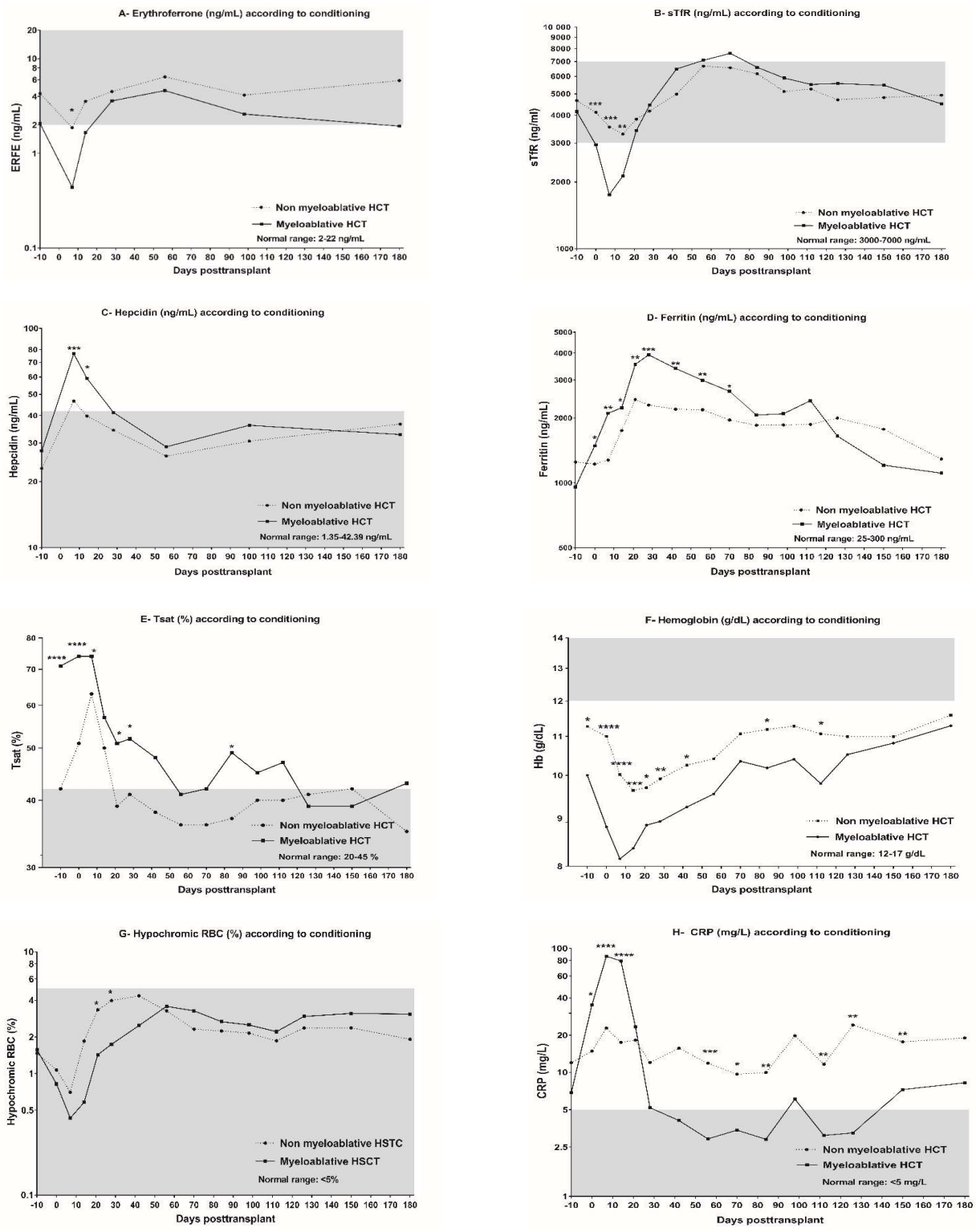


**Figure 4:** Temporal evolution of mean erythroferrone (A), soluble transferrin receptor (B), hepcidin (C), ferritin (D), transferrin saturation (E), hemoglobin (F), hypochromic red blood cells (G) and CRP (H) following HSCT (day 0), in patients after myeloablative (n=24) or nonmyeloablative (n=46) conditioning. *: < 0.05; **: < 0.01; ***: < 0.001; ****: < 0.0001. The gray zone represents the reference interval in normal subjects.

### 1.3.2 Factors associated with serum hepcidin levels

**In univariate analyses,** hepcidin at any time point correlated well with values at nearly all later time points (p<0.0001). All hepcidin values also correlated with preceding or subsequent SF levels (r = 0.39 to 0.74, p<0.0001). Hepcidin also correlated positively with Tsat (except at day 7; r=0.25 to 0.36, p=0.04 to 0.002), CRP (from day -10 to day 14: r=0.23 to 0.45, p=0.046 to 0.0001), and inversely with sTfR (from day -10 to day 28, r=−0.43 to −0.48, p=0.0002 to < 0.0001), ERFE (on day 14, r=−0.29, p=0.02), Hb (from day -10 to day 28, r=−0.25 to −0.52, p=0.04 to <0.0001) and reticulocytes (from day -10 to day 28, r=−0.26 to −0.40, p=0.03 to 0.0005) at early time points. Negative correlations were also observed between hepcidin and platelets (from day -10 to day 100: r=-0.25 to -0.42, p=0.04 to 0.0003), and WBC (from day -10 to day 14: r =-0.27 to -0.32, p=0.03 to 0.007), but not serum EPO at day 28, creatinine, uric acid, ASAT, bilirubin or LDH. **Figure 5** displays the most significant overall correlations, comprising all time points, between hepcidin and SF (**Figure 5A**), Tsat (**Figure 5B**), ERFE (**Figure 5C**), sTfR (**Figure 5D**), reticulocytes (**Figure 5E**) or Hb (**Figure 5F**). Finally, based on the median number of 4 RBC transfusions received between days 0 and 100, we compared hepcidin levels in patients receiving 0-4 vs >4 RBC transfusions. Hepcidin was significantly higher in patients who received >4 RBC transfusions (**Figure 6**).

**In multivariate linear regressions**, number of RBC transfusions (β-coefficient=0.11, p=0.02) and pretransplant SF (β-coefficient=0.29, p=0.001) explained 52 % of the variation of pretransplant hepcidin ($R^2$=0.52, p<0.0001). After transplantation, iron (SF at all time points except day 7 (β-coefficient between 0.38 and 0.65, p<0.0001), Tsat at day 56 (β-coefficient=-0.62, p=0.0003)) and erythropoietic parameters (reticulocytes at day 7 (β-coefficient=-0.10, p=0.004) and 100 (β-coefficient=-0.05, p=0.05), ERFE at day 14 (β-coefficient=-0.23, p=0.0001), and sTfR at days 28 (β-coefficient=-0.27, p=0.02) and 56 (β-coefficient=-0.38, p=0.006)) were the most predictive factors of hepcidin levels (R2=0.36 to 0.60, p<0.0001). Age, sex, diagnosis, DRI, HCT-CI, PS, conditioning, donor type, graft type, ABO, platelet transfusions, CMV status, GVHD prophylaxis, GVHD, infections and EPO treatment did not contribute to this prediction.

### 1.3.3 Factors associated with serum ERFE levels

**In univariate analyses**, ERFE at any time point correlated only with ERFE values at the next 2 time points. During conditioning and engraftment until day 14, all ERFE values correlated with preceding, same day or subsequent sTfR levels (r=0.27 to 0.45, p=0.03 to 0.0002) and reticulocyte counts (except day 28; r=0.27 to 0.61, p=0.05 to <0.0001) and the correlations were strong on same day samples considering all time points together for sTfR (r=0.31, p<0.0001, Figure 3G) and reticulocytes (r=0.40, p<0.0001, Figure 3H). After engraftment, ERFE correlated negatively with Hb (r=-0.38, p=0.002 on day 100; r=-0.35, p=0.01 on day 180). The overall correlation between

same day ERFE and hepcidin was weak (r=-0.13, p=0.0095, **Figure 5C**). We also observed correlations between ERFE and LDH (from day 14 to day 180: r=0.28 to 0.39, p=0.03 to 0.009), WBC (from day 7 to day 14: r=0.25 to 0.45, p=0.05 to 0.0003), platelets (on days 100 (r=-0.28, p=0.03) and 180 (r=-0.33, p=0.03)), but not with SF, CRP, renal or liver parameters. ERFE was higher in males compares to females overall (p=0.03), but this was true only in the control arm (p=0.01) and not in the EPO arm. ERFE was significantly lower in patients with >4 RBC transfusions between days 0 and 100, but only at days 7 and 14 (**Figure 6**). This may be explained by the fact that patients with low transfusion needs were less anemic (11.5±2.0 vs 10.0±2.0 gr/dL) before transplantation and more frequently received NMA conditioning (only 4 patients with MA among the 36 patients with <4 transfusions) and therefore had less suppression of erythropoiesis. **In multivariate analyses**, the major determinant of ERFE during conditioning and engraftment (R2=0.18 to 0.39, p=0.0008 to <0.0001) was erythropoietic activity as measured by sTfR (at days 7 (β-coefficient=0.69, p=0.0008) or 100 (β-coefficient=0.73, p=0.0029)), reticulocytes (at days -10 (β-coefficient=0.16, p=0.001), 14 (β-coefficient=0.14, p=0.004) or 100 (β-coefficient=0.20, p<0.0001)) or day 28 serum EPO (β-coefficient=0.45, p=0.0032). This is also illustrated by the relatively parallel kinetics of ERFE and sTfR levels (Figure 1). At day 180, ERFE correlated mostly with neutrophils (β-coefficient=0.75, p=0.0042) and negatively with Tsat (β-coefficient=-0.88, p=0.0009). ERFE correlated with male sex pretransplant (β-coefficient=0.68, p=0.03) and at day 14 (β-coefficient=0.81, p=0.02). The other clinical parameters listed for hepcidin did not contribute to prediction of ERFE values.

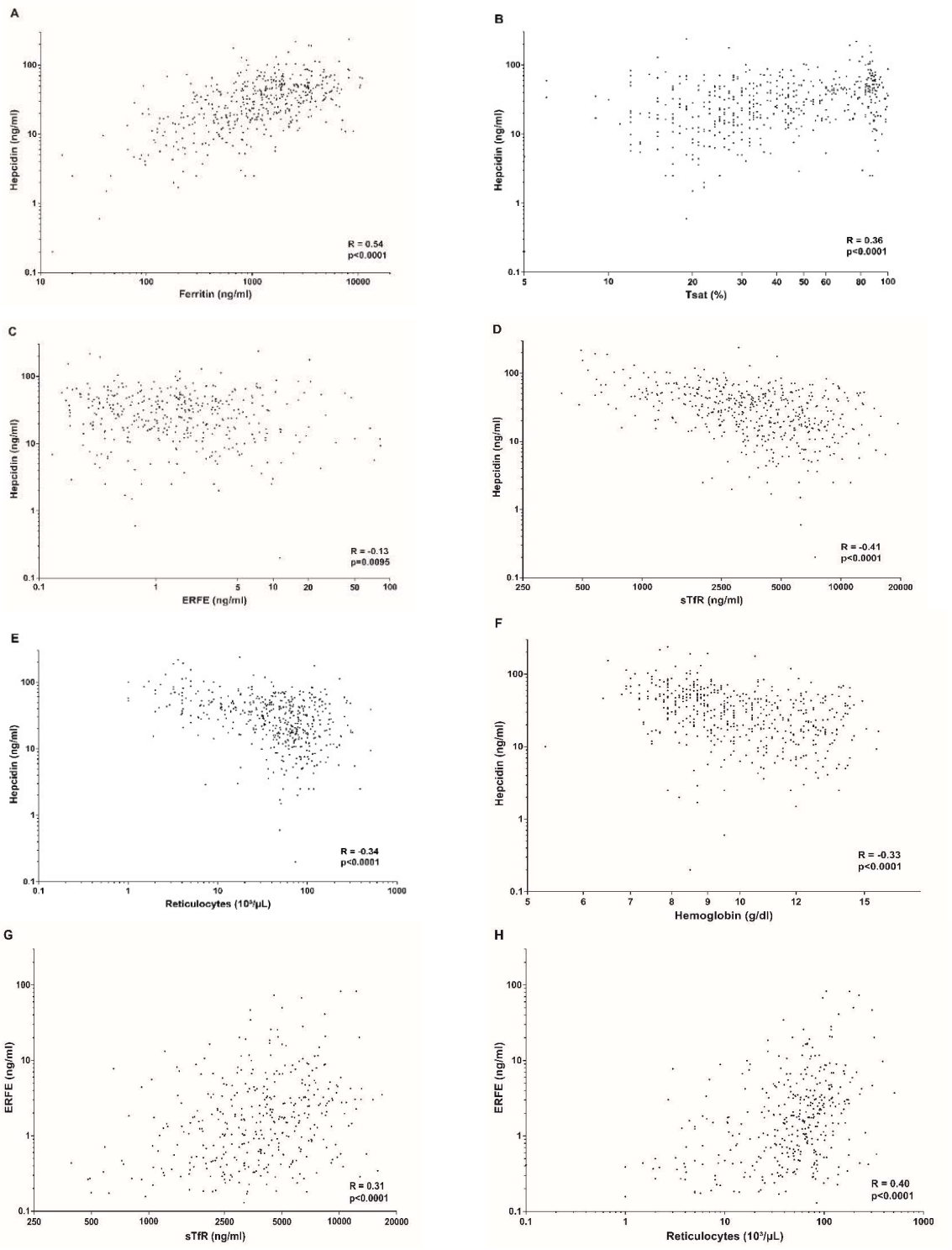


**Figure 5:** Correlations between same day hepcidin or ERFE and parameters of iron metabolism and erythropoiesis in the whole group of patients, each of them being sampled at all time points after allo-HSCT. [A-F] Hepcidin correlated strongly with (A) ferritin (R = 0.54, p < 0.0001), (B) Tsat (R = 0.36, p<0.0001), (C) ERFE (R = -0.13, p=0.0095), (D) sTfR (R = -0.41, p<0.0001), (E) reticulocytes (R = -0.34, p<0.0001) or (F) Hb (R = -0.33, p<0.0001). [G-H] ERFE correlated strongly with (G) sTfR (R = 0.31, p<0.0001) or (H) reticulocytes (R = 0.40, p<0.0001).

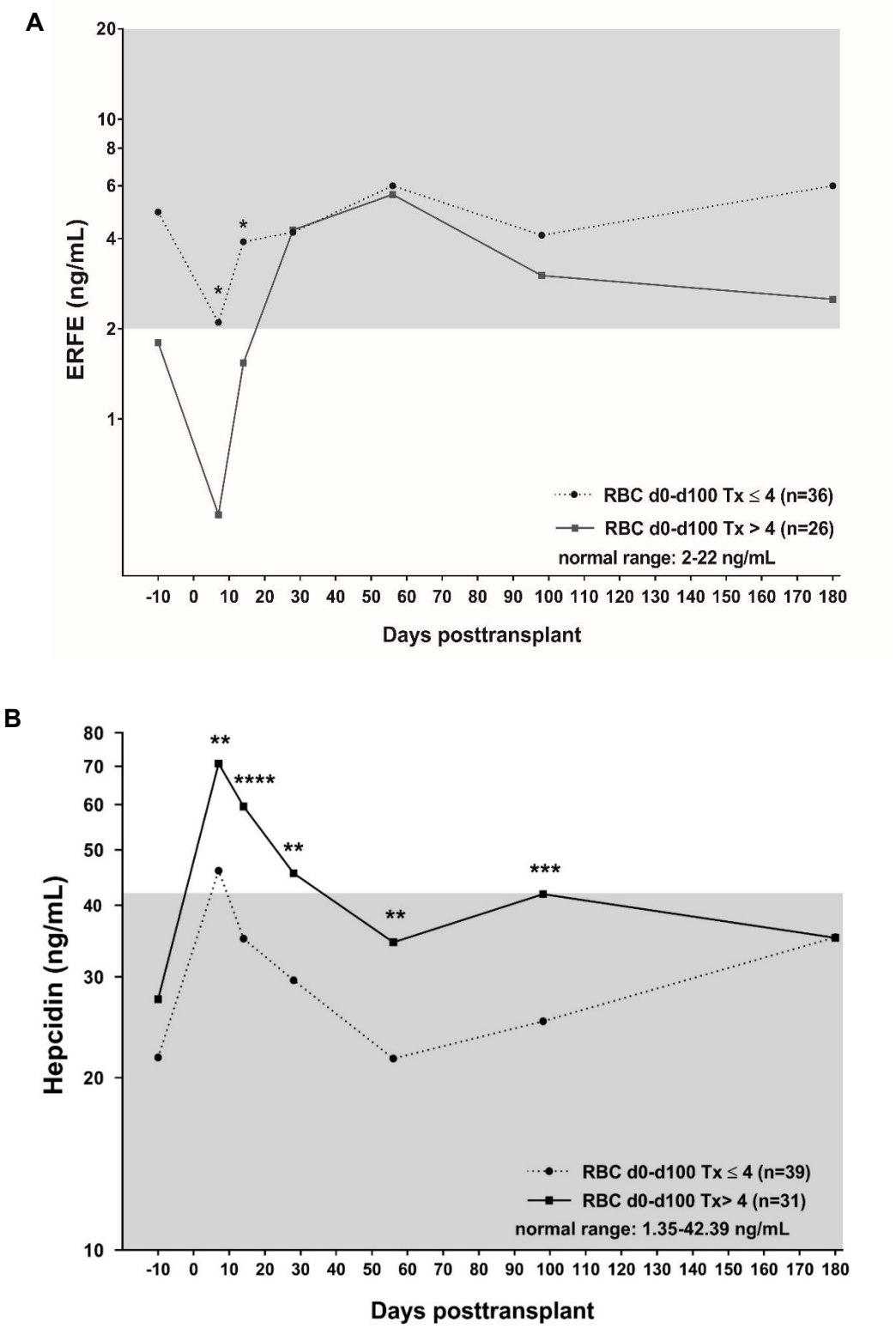


**Figure 6**: Temporal evolution of mean ERFE (A) and hepcidin (B) following HSCT (day 0) according to number of RBC transfusions received from day 0 until day +100 (the cutoff for number of RBC transfusions was the median value of 4 RBC transfusions received between day 0 and day +100), in all patients. *: < 0.05; **: < 0.01; ***: < 0.001; ****: < 0.0001. The gray zone represents the reference interval in normal subjects.

## 1.4 Discussion

Few studies have examined the significance of serum hepcidin in the context of allogeneic HSCT (53-57) and none has analyzed in detail the interplay between iron metabolism and erythropoietic activity in this setting. In 2014, Kautz identified ERFE, an erythropoietic regulator of iron mobilization and absorption through hepcidin suppression (18). Subsequently, studies demonstrated increased serum ERFE low serum hepcidin in subjects living at high altitude and healthy subjects after EPO injection (58, 59). This prompted us to investigate ERFE regulation and role in the pathophysiology of iron metabolism and erythropoiesis after allo-HSCT. A randomized trial of EPO therapy (41) provided a further opportunity to investigate the interactions between iron, inflammation and erythropoietic activity over time after transplantation.

After destruction of erythroid precursors following conditioning, marrow erythroid activity is strongly suppressed (24, 60). Indeed, we observed that erythropoiesis decreased consistently, and as expected more severely so after MA than NMA conditioning. Both myelosuppression and defective EPO production could contribute, as we previously showed that endogenous EPO remained appropriate for the degree of anemia after NMA conditioning but rapidly became inadequately low after MA transplantation (25). Hb, reticulocytes, hypochromic red cells and sTfR decreased sharply and, in addition, we demonstrated for the first time that this also led to a significant drop in ERFE levels. Suppression of erythropoiesis resulted in a significant peak in Tsat values. The close relationship between erythropoietic activity and serum ERFE is also illustrated by significant correlations between ERFE and sTfR or reticulocytes, as well as by the relative parallelism between sTfR and ERFE evolutions from before conditioning through six months posttransplant. Although sTfR and ERFE have similar time course (**Figure 1A and 1B**), there is a striking difference between the response of sTfR and ERFE to exogenous EPO. EPO therapy per se did not translate into further enhancement of ERFE as shown in healthy subjects (59). One likely explanation is that sTfR (but not ERFE) also responds to EPO-induced functional iron deficiency, as illustrated by decreasing transferrin saturation and the appearance of hypochromic red cells (**Figure 1E and 1G**), sensed by erythroblasts. Moreover, it is possible that, contrarily to EPO therapy in normal individuals with stable erythropoiesis, EPO treatment in the setting of a dynamically changing erythropoietic environment after allo-HSCT, with large inter-patient variations, may not produce further statistically significant modifications in ERFE production rates. Finally, it could be speculated that nascent erythroblasts in an altered environment could have an inappropriate secretion of ERFE upon EPO injection or that still unknown inhibitory factors of ERFE secretion may operate in the setting of allo-HSCT, but this remains to be investigated.

We also observed a strong surge of SF values culminating on day 42 in the control arm and 28 in the EPO arm. This SF peak has been observed previously (61, 62) and may be explained in part by

inactive erythropoiesis resulting in decreased marrow demand for iron, Tsat elevation by non-utilization of SeFe, and further iron deposition in tissues. However, the SF peak was delayed relative to engraftment. To explain that other factors must be involved, such as conditioning-induced inflammation and hepatic cytolysis (63). Indeed, there was an early, moderate, rise in ASAT and a later increase of LDH (**Figure 2D and 2C**), but a relationship with the SF course is not evident. On the other hand, CRP also peaked very early (**Figure 1H**) but its normalization did not prevent SF from further increasing. CRP remained stable after NMA conditioning (**Figure 4H**), but this did not prevent SF from raising in this setting also (**Figure 4D**). Serum albumin decreased until day 14 and slowly recovered thereafter (**Figure 2B**), also preceding SF changes. We have not measured other, more specific, markers of inflammation. Of course, IO worsens posttransplant with RBC transfusions, but only 4 patients received IV iron and none before day 87, excluding IV iron as a culprit. On the other hand, compared to histocompatible HSCT, mice given histoincompatible T cells show a loss of expression of hepatic hepcidin, enhancing iron absorption, rising SeFe and contributing to liver IO after allo-HSCT (64). Therefore, the pathophysiology of SF kinetics after transplantation remains very complex and this study lacks the power to dissect all these potential explanatory pathways. Hence, we are engaged in another study specifically addressing this issue in a group of >500 patients.

Pretransplant hepcidin was strongly associated with the number of previous RBC transfusions and SF. Paralleling the kinetics of SF and Tsat and inversely following those of ERFE and sTfR, hepcidin increased significantly over baseline and peaked at day 7. This increase could be explained by hepcidin upregulation from inflammation and IO through the BMP complex and SMAD pathway (5) and through downregulation of ERFE production following post-conditioning myelosuppression (19). This also supports the experimental evidence that suppression of hepcidin during anemia requires erythropoietic activity (22, 65). Kanda (54) attributed a similar SF peak after auto-SCT to inflammation because of a concomitant elevation of serum IL-6 levels (not measured in our study), but, although CRP also peaked at day 7, we did not observe any correlation between hepcidin and CRP values.

After day 7, hepcidin progressively returned to baseline, despite persistence of very high SF values. Although this was similar with or without EPO therapy, it suggests that engraftment and erythropoietic recovery were capable of a certain degree of regulation of hepcidin production. Hepcidin at all time points correlated positively with SF and Tsat and inversely with sTfR, ERFE, Hb and reticulocytes. In addition, there was a strong overall correlation between hepcidin and SF ($r=0.54$, $p<0.0001$) and a much weaker negative correlation with ERFE ($r=-0.13$, $p=0.0095$). Furthermore, multivariate disclosed very strong associations between hepcidin and SF from day 14 through day 180 after HSCT, and weaker associations with erythropoietic markers. This could indicate that, contrarily to thalassemia where negative erythropoietic regulators dominate

positive iron regulators of hepcidin production (21), the reverse is true after allo-HSCT even with EPO therapy. This apparently contradicts our previous study after auto-HSCT (22), where EPO therapy induced stronger inhibition of hepcidin production than in the current study. However, separate analyses of patients with low and high baseline SF (Figure 2) showed that EPO therapy modulated hepcidin levels when iron burden was low (as in auto-HSCT), but also indicated that the hepcidin-inducing ability of IO in heavily transfused patients with myeloid malignancies (66) was capable to annihilate erythroid regulation of hepcidin by EPO therapy. Likewise, hepcidin was significantly higher and ERFE lower in patients receiving >4 RBC transfusions between days 0 and 100. These were patients who arrived more anemic at transplantation and received more frequently MA conditioning, but this additional iron burden also contributed to higher hepcidin values.

Furthermore, similar sTfR kinetics in patients with low or high baseline SF suggest that this is not due to an impact of iron burden on response to EPO (**Figure 3E and 3F**). Serum sTfR depends on the number of cells bearing TfR receptors (elevated with increased erythropoietic activity, for instance with EPO therapy (**Figure 3D**) and on TfR receptor density on cells (elevated in iron deficiency but not inflammation). Thus, sTfR is a good quantitative marker of erythropoietic activity (high positive correlation between sTfR and Hb, **Figure 7**) and, to a lesser extent, iron status (67).

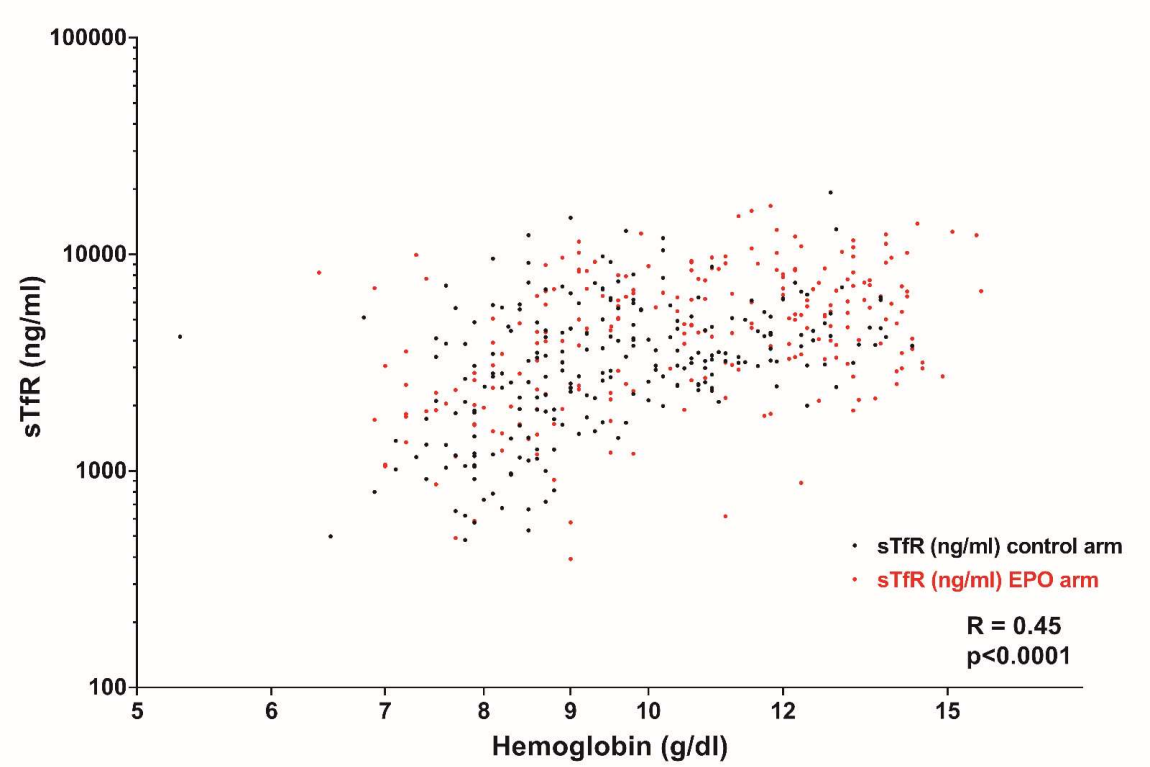


**Figure 7:** Correlations between same day sTfR and hemoglobin at all time points in the control (black points; R = 0.43, p<0.0001) and EPO (red points; R = 0.41, p=0.0001) arms (overall R = 0.45, p<0.0001).

In conclusion, we investigated hepcidin regulation in the setting of allo-HSCT with a variety of parameters of iron metabolism and erythropoiesis. The kinetics and correlations of ERFE, hepcidin and sTfR after transplantation confirm the strong regulatory effects of erythropoiesis and iron status on posttransplant hepcidin. However, contrarily to other situations, the inhibitory effect of IO appears to dominate the stimulating effect of erythropoietic activity on hepcidin production. Inflammation did not appear to exert a major effect on hepcidin. However, besides CRP, which is not well coordinated among the overall patient population as it may vary abruptly in individual patients, inflammation was only assessed by a limited number of less specific markers. For example, Kanda demonstrated an elevation of serum IL-6 levels concomitantly with the SF peak (54). Another limitation in this study is potential collinearity in multivariable analyses. Indeed, some explanatory variables are highly correlated with each other, and therefore only some remain significant in multivariate analyzes.

The study also supports the role of erythropoiesis in ERFE production (18, 59). Investigations of hepcidin and ERFE, in addition to the more classical parameters of iron metabolism and erythropoiesis, may shed additional light on the interplay between erythropoiesis and iron metabolism during allo-HSCT as well as in other settings.

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## 1.5 References

1. Beguin Y, Aapro M, Ludwig H, Mizzen L, Osterborg A. Epidemiological and nonclinical studies investigating effects of iron in carcinogenesis--a critical review. Crit Rev Oncol Hematol. 2014;89(1):1-15.
2. Qiao B, Sugianto P, Fung E, Del-Castillo-Rueda A, Moran-Jimenez MJ, Ganz T, et al. Hepcidin-induced endocytosis of ferroportin is dependent on ferroportin ubiquitination. Cell Metab. 2012;15(6):918-24.
3. Camaschella C, Nai A, Silvestri L. Iron metabolism and iron disorders revisited in the hepcidin era. Haematologica. 2020;105(2):260-72.
4. Aschemeyer S, Qiao B, Stefanova D, Valore EV, Sek AC, Ruwe TA, et al. Structure-function analysis of ferroportin defines the binding site and an alternative mechanism of action of hepcidin. Blood. 2018;131(8):899-910.
5. Ganz T. Hepcidin and iron regulation, 10 years later. Blood. 2011;117(17):4425-33.
6. Canali S, Wang CY, Zumbrennen-Bullough KB, Bayer A, Babitt JL. Bone morphogenetic protein 2 controls iron homeostasis in mice independent of Bmp6. Am J Hematol. 2017;92(11):1204-13.
7. Meynard D, Kautz L, Darnaud V, Canonne-Hergaux F, Coppin H, Roth MP. Lack of the bone morphogenetic protein BMP6 induces massive iron overload. Nat Genet. 2009;41(4):478-81.
8. Nai A, Rubio A, Campanella A, Gourbeyre O, Artuso I, Bordini J, et al. Limiting hepatic Bmp-Smad signaling by matriptase-2 is required for erythropoietin-mediated hepcidin suppression in mice. Blood. 2016;127(19):2327-36.
9. Jaspers A, Caers J, Le Gac G, Ferec C, Beguin Y, Fillet G. A novel mutation in the CUB sequence of matriptase-2 (TMPRSS6) is implicated in iron-resistant iron deficiency anaemia (IRIDA). Br J Haematol. 2013;160(4):564-5.
10. Gao J, Chen J, Kramer M, Tsukamoto H, Zhang AS, Enns CA. Interaction of the hereditary hemochromatosis protein HFE with transferrin receptor 2 is required for transferrin-induced hepcidin expression. Cell Metab. 2009;9(3):217-27.
11. Armitage AE, Eddowes LA, Gileadi U, Cole S, Spottiswoode N, Selvakumar TA, et al. Hepcidin regulation by innate immune and infectious stimuli. Blood. 2011;118(15):4129-39.
12. Mayeur C, Lohmeyer LK, Leyton P, Kao SM, Pappas AE, Kolodziej SA, et al. The type I BMP receptor Alk3 is required for the induction of hepatic hepcidin gene expression by interleukin-6. Blood. 2014;123(14):2261-8.
13. Liu Q, Davidoff O, Niss K, Haase VH. Hypoxia-inducible factor regulates hepcidin via erythropoietin-induced erythropoiesis. J Clin Invest. 2012;122(12):4635-44.
14. Sasaki Y, Noguchi-Sasaki M, Yasuno H, Yorozu K, Shimonaka Y. Erythropoietin stimulation decreases hepcidin expression through hematopoietic activity on bone marrow cells in mice. Int J Hematol. 2012;96(6):692-700.
15. Ashby DR, Gale DP, Busbridge M, Murphy KG, Duncan ND, Cairns TD, et al. Erythropoietin administration in humans causes a marked and prolonged reduction in circulating hepcidin. Haematologica. 2010;95(3):505-8.
16. Weiss G, Theurl I, Eder S, Koppelstaetter C, Kurz K, Sonnweber T, et al. Serum hepcidin concentration in chronic haemodialysis patients: associations and effects of dialysis, iron and erythropoietin therapy. Eur J Clin Invest. 2009;39(10):883-90.
17. Pinto JP, Ribeiro S, Pontes H, Thowfeequ S, Tosh D, Carvalho F, et al. Erythropoietin mediates hepcidin expression in hepatocytes through EPOR signaling and regulation of C/EBPalpha. Blood. 2008;111(12):5727-33.

18. Kautz L, Jung G, Valore EV, Rivella S, Nemeth E, Ganz T. Identification of erythroferrone as an erythroid regulator of iron metabolism. Nat Genet. 2014;46(7):678-84.
19. Arezes J, Foy N, McHugh K, Sawant A, Quinkert D, Terraube V, et al. Erythroferrone inhibits the induction of hepcidin by BMP6. Blood. 2018;132(14):1473-7.
20. Lasocki S, Millot S, Andrieu V, Lettéron P, Pilard N, Muzeau F, et al. Phlebotomies or erythropoietin injections allow mobilization of iron stores in a mouse model mimicking intensive care anemia. Crit Care Med. 2008;36(8):2388-94.
21. Nemeth E. Hepcidin and β-thalassemia major. Blood. 2013;122(1):3-4.
22. Jaspers A, Baron F, Willems E, Seidel L, Wiegerinck ET, Swinkels DW, et al. Serum hepcidin following autologous hematopoietic cell transplantation: an illustration of the interplay of iron status, erythropoiesis and inflammation. Haematologica. 2014;99(3):e35-7.
23. Beguin Y, Baron F, Fillet G. Influence of marrow erythropoietic activity on serum erythropoietin levels after autologous hematopoietic stem cell transplantation. Haematologica. 1998;83(12):1076-81.
24. Beguin Y, Oris R, Fillet G. Dynamics of erythropoietic recovery following bone marrow transplantation: role of marrow proliferative capacity and erythropoietin production in autologous versus allogeneic transplants. Bone Marrow Transplant. 1993;11(4):285-92.
25. Baron F, Fillet G, Beguin Y. Erythropoiesis after nonmyeloablative stem-cell transplantation is not impaired by inadequate erythropoietin production as observed after conventional allogeneic transplantation. Transplantation. 2002;74(12):1692-6.
26. Beguin Y, Clemons GK, Oris R, Fillet G. Circulating erythropoietin levels after bone marrow transplantation: inappropriate response to anemia in allogeneic transplants. Blood. 1991;77(4):868-73.
27. Beguin Y, Yerna M, Loo M, Weber M, Fillet G. Erythropoiesis in multiple myeloma: defective red cell production due to inappropriate erythropoietin production. Br J Haematol. 1992;82(4):648-53.
28. Cazzola M, Guarnone R, Cerani P, Centenara E, Rovati A, Beguin Y. Red blood cell precursor mass as an independent determinant of serum erythropoietin level. Blood. 1998;91(6):2139-45.
29. Beguin Y, Clemons GK, Pootrakul P, Fillet G. Quantitative assessment of erythropoiesis and functional classification of anemia based on measurements of serum transferrin receptor and erythropoietin. Blood. 1993;81(4):1067-76.
30. Beguin Y, Maertens J, De Prijck B, Schots R, Seidel L, Bonnet C, et al. Darbepoetin-alfa and intravenous iron administration after autologous hematopoietic stem cell transplantation: a prospective multicenter randomized trial. Am J Hematol. 2013;88(12):990-6.
31. Locatelli F, Zecca M, Pedrazzoli P, Prete L, Quaglini S, Comoli P, et al. Use of recombinant human erythropoietin after bone marrow transplantation in pediatric patients with acute leukemia: effect on erythroid repopulation in autologous versus allogeneic transplants. Bone Marrow Transplant. 1994;13(4):403-10.
32. Baron F, Sautois B, Baudoux E, Matus G, Fillet G, Beguin Y. Optimization of recombinant human erythropoietin therapy after allogeneic hematopoietic stem cell transplantation. Exp Hematol. 2002;30(6):546-54.
33. Jaspers A, Baron F, Maertens J, De Prijck B, Schots R, Bonnet C, et al. Long-term safety follow-up of a randomized trial of darbepoetin alpha and intravenous iron following autologous hematopoietic cell transplantation. Am J Hematol. 2015;90(7):E133-4.
34. Cavill I, Auerbach M, Bailie GR, Barrett-Lee P, Beguin Y, Kaltwasser P, et al. Iron and the anaemia of chronic disease: a review and strategic recommendations. Curr Med Res Opin. 2006;22(4):731-7.

35. Aapro M, Österborg A, Gascón P, Ludwig H, Beguin Y. Prevalence and management of cancer-related anaemia, iron deficiency and the specific role of i.v. iron. Ann Oncol. 2012;23(8):1954-62.
36. Beguin Y. Prediction of response to optimize outcome of treatment with erythropoietin. Semin Oncol. 1998;25(3 Suppl 7):27-34.
37. Beguin Y. Prediction of response and other improvements on the limitations of recombinant human erythropoietin therapy in anemic cancer patients. Haematologica. 2002;87(11):1209-21.
38. Beguin Y, Loo M, R'Zik S, Sautois B, Lejeune F, Rorive G, et al. Early prediction of response to recombinant human erythropoietin in patients with the anemia of renal failure by serum transferrin receptor and fibrinogen. Blood. 1993;82(7):2010-6.
39. Jaspers A, Baron F, Servais S, Lejeune M, Willems É, Seidel L, et al. Erythropoietin therapy after allogeneic hematopoietic cell transplantation has no impact on long-term survival. Am J Hematol. 2015;90(9):E197-9.
40. Aapro M, Beguin Y, Bokemeyer C, Dicato M, Gascón P, Glaspy J, et al. Management of anaemia and iron deficiency in patients with cancer: ESMO Clinical Practice Guidelines. Ann Oncol. 2018;29(Suppl 4):iv271.
41. Jaspers A, Baron F, Willems E, Seidel L, Hafraoui K, Vanstraelen G, et al. Erythropoietin therapy after allogeneic hematopoietic cell transplantation: a prospective, randomized trial. Blood. 2014;124(1):33-41.
42. Armand P, Kim HT, Logan BR, Wang Z, Alyea EP, Kalaycio ME, et al. Validation and refinement of the Disease Risk Index for allogeneic stem cell transplantation. Blood. 2014;123(23):3664-3671
43. Sorror ML, Maris MB, Storb R, Baron F, Sandmaier BM, Maloney DG, et al. Hematopoietic cell transplantation (HCT)-specific comorbidity index: a new tool for risk assessment before allogeneic HCT. Blood. 2005;106(8):2912-2919.
44. Bovy C, Tsobo C, Crapanzano L, Rorive G, Beguin Y, Albert A, et al. Factors determining the percentage of hypochromic red blood cells in hemodialysis patients. Kidney Int. 1999;56(3):1113-9.
45. Bovy C, Gothot A, Delanaye P, Warling X, Krzesinski JM, Beguin Y. Mature erythrocyte parameters as new markers of functional iron deficiency in haemodialysis: sensitivity and specificity. Nephrol Dial Transplant. 2007;22(4):1156-62.
46. Beguin Y. The soluble transferrin receptor: biological aspects and clinical usefulness as quantitative measure of erythropoiesis. Haematologica. 1992;77(1):1-10.
47. R'zik S, Beguin Y. Serum soluble transferrin receptor concentration is an accurate estimate of the mass of tissue receptors. Exp Hematol. 2001;29(6):677-85.
48. R'zik S, Loo M, Beguin Y. Reticulocyte transferrin receptor (TfR) expression and contribution to soluble TfR levels. Haematologica. 2001;86(3):244-51.
49. Cazzola M, Beguin Y, Bergamaschi G, Guarnone R, Cerani P, Barella S, et al. Soluble transferrin receptor as a potential determinant of iron loading in congenital anaemias due to ineffective erythropoiesis. Br J Haematol. 1999;106(3):752-5.
50. Diepeveen LE, Laarakkers CMM, Martos G, Pawlak ME, Uğuz FF, Verberne KESA, et al. Provisional standardization of hepcidin assays: creating a traceability chain with a primary reference material, candidate reference method and a commutable secondary reference material. Clin Chem Lab Med. 2019;57(6):864-72.
51. Beguin Y, Lipscei G, Oris R, Thoumsin H, Fillet G. Serum immunoreactive erythropoietin during pregnancy and in the early postpartum. Br J Haematol. 1990;76(4):545-9.

52. Corazza F, Beguin Y, Bergmann P, André M, Ferster A, Devalck C, et al. Anemia in children with cancer is associated with decreased erythropoietic activity and not with inadequate erythropoietin production. Blood. 1998;92(5):1793-8.
53. Kanda J, Mizumoto C, Kawabata H, Ichinohe T, Tsuchida H, Tomosugi N, et al. Clinical significance of serum hepcidin levels on early infectious complications in allogeneic hematopoietic stem cell transplantation. Biol Blood Marrow Transplant. 2009;15(8):956-62.
54. Kanda J, Mizumoto C, Kawabata H, Tsuchida H, Tomosugi N, Matsuo K, et al. Serum hepcidin level and erythropoietic activity after hematopoietic stem cell transplantation. Haematologica. 2008;93(10):1550-4.
55. Eisfeld AK, Westerman M, Krahl R, Leiblein S, Liebert UG, Hehme M, et al. Highly Elevated Serum Hepcidin in Patients with Acute Myeloid Leukemia prior to and after Allogeneic Hematopoietic Cell Transplantation: Does This Protect from Excessive Parenchymal Iron Loading? Adv Hematol. 2011;2011:491058.
56. Sakamoto S, Kawabata H, Kanda J, Uchiyama T, Mizumoto C, Kitano T, et al. High pretransplant hepcidin levels are associated with poor overall survival and delayed platelet engraftment after allogeneic hematopoietic stem cell transplantation. Cancer Med. 2017;6(1):120-8.
57. Li X, Xu F, Karoopongse E, Marcondes AM, Lee K, Kowdley KV, et al. Allogeneic transplantation, Fas signaling, and dysregulation of hepcidin. Biol Blood Marrow Transplant. 2013;19(8):1210-9.
58. Ganz T, Jung G, Naeim A, Ginzburg Y, Pakbaz Z, Walter PB, et al. Immunoassay for human serum erythroferrone. Blood. 2017;130(10):1243-6.
59. Robach P, Gammella E, Recalcati S, Girelli D, Castagna A, Roustit M, et al. Induction of erythroferrone in healthy humans by micro-dose recombinant erythropoietin or high-altitude exposure. Haematologica. 2020.
60. Vellenga E, Sizoo W, Hagenbeek A, Löwenberg B. Different repopulation kinetics of erythroid (BFU-E), myeloid (CFU-GM) and T lymphocyte (TL-CFU) progenitor cells after autologous and allogeneic bone marrow transplantation. Br J Haematol. 1987;65(2):137-42.
61. Fingrut W, Law A, Lam W, Michelis FV, Viswabandya A, Lipton JH, et al. Post-transplant ferritin level predicts outcomes after allogeneic hematopoietic stem cell transplant, independent from pre-transplant ferritin level. Ann Hematol. 2021.
62. Or R, Matzner Y, Konijn AM. Serum ferritin in patients undergoing bone marrow transplantation. Cancer. 1987;60(5):1127-31.
63. Gordon LI, Brown SG, Tallman MS, Rademaker AW, Weitzman SA, Lazarus HM, et al. Sequential changes in serum iron and ferritin in patients undergoing high-dose chemotherapy and radiation with autologous bone marrow transplantation: possible implications for treatment related toxicity. Free Radic Biol Med. 1995;18(3):383-9.
64. Bair S, Spaulding E, Parkkinen J, Shulman HM, Lesnikov V, Beauchamp M, et al. Transplantation of allogeneic T cells alters iron homeostasis in NOD/SCID mice. Blood. 2009;113(8):1841-4.
65. Pak M, Lopez MA, Gabayan V, Ganz T, Rivera S. Suppression of hepcidin during anemia requires erythropoietic activity. Blood. 2006;108(12):3730-5.
66. Alessandrino EP, Della Porta MG, Bacigalupo A, Malcovati L, Angelucci E, Van Lint MT, et al. Prognostic impact of pre-transplantation transfusion history and secondary iron overload in patients with myelodysplastic syndrome undergoing allogeneic stem cell transplantation: a GITMO study. Haematologica. 2010;95(3):476-84.
67. Beguin Y. Soluble transferrin receptor for the evaluation of erythropoiesis and iron status. Clin Chim Acta. 2003;329(1-2):9-22.

## 2. Factors influencing pretransplant serum ferritin and hepcidin levels and their impact on outcomes after allogeneic hematopoietic stem cell transplantation

## Abstract

**Objectives:** Elevated pre-transplant serum ferritin (SF) levels have been associated with reduced overall survival (OS) and progression-free survival (PFS) as well as an increased risk of infections. Hepcidin, the regulator of iron metabolism, has been minimally studied after allogeneic hematopoietic stem cell transplantation (allo-HSCT), with an uncertain impact on survival and infections. We explore the association between pretransplant SF and hepcidin with OS, PFS, relapse, non-relapse mortality (NRM), infections, engraftment, and graft-versus-host disease (GVHD) in 502 patients.

**Materials and Methods**: 502 consecutive patients who underwent allo-HSCT at the University Hospital of Liège between 1999 and 2012 and had a minimal follow-up of 5 years were included. Pretransplant serum hepcidin (measured by mass spectrometry) and SF levels were investigated using univariate and multivariate linear regression. Patients were grouped into quartiles based on their values. OS, PFS, relapse, engraftment, infections, and GVHD were estimated using the Kaplan-Meier method, compared between subgroups (log-rank test), and analyzed in multivariate Cox models. Cumulative incidence of NRM was estimated by standard methods, with relapse and progression treated as competing risks.

**Results:** Median pretransplant hepcidin levels was 25.13 ng/mL, and SF 1,083 ng/mL, with a median SF peak of 3,419 ng/mL within the first 100 days post-transplantation. High SF, CRP, pretransplant red blood cell (RBC) transfusions, HCT-CI, performance status (PS) and low neutrophils were independently associated with elevated pretransplant hepcidin ($R^2$ = 0.44, $p<0.0001$). Pre-transplant SF levels were positively associated with pre-transplant hepcidin, transferrin saturation, alanine aminotransferase, creatinine, pretransplant RBC and platelet transfusions, PS, negatively correlated with Hb levels and influenced by diagnostic type ($R^2$=0.67, $p<0.0001$). OS and PFS were significantly lower in the highest hepcidin ($p<0.0001$ & $p=0.0006$) and SF ($p<0.0001$ & $p=0.0003$) groups compared to groups with lower values. The predictive power of SF surpassed that of hepcidin on survival outcomes in UV analysis. In MV analysis, pretransplant SF was significantly correlated with decreased survival, but hepcidin was not. Elevated pretransplant SF was also associated with an increased risk of post-transplant infections in UV but not in MV analysis, yielding to transferrin saturation (Tsat), while hepcidin did not have a significant effect on infections. SF, but not hepcidin, correlated with slower engraftment in UV but not in MV analysis. No significant association was observed between the 2 parameters and GVHD.

**Discussion**: The significance of pretransplant hepcidin and SF levels is complex. Pretransplant SF and hepcidin levels not only serve as markers of iron overload due to prior transfusions but also depend on other factors reflecting patient comorbidities (inflammation, renal insufficiency, hepatic cytolysis, anemia, and overall comorbidities) and the underlying hematologic disease. Elevated pretransplant SF levels are associated with adverse outcomes, including reduced OS, PFS and NRM and an increased risk of infections and poor engraftment. However, it largely loses its predictive power in multivariate analysis.

**Conclusion**: Elevated pretransplant SF levels are more strongly associated with adverse post-transplant outcomes than increased hepcidin. This study highlights the complexity of iron metabolism and its impact on outcomes after allo-HSCT, underscoring the need to consider multiple biological and clinical variables in transplantation prognosis.

## 2.1 Introduction

Allo-HSCT is an important treatment modality for various malignant and nonmalignant diseases. Nevertheless, transplantation-related morbidity and mortality is considerable, the most frequent being infections, conditioning regimen–related toxicities and GVHD (1). This mortality is influenced by several factors, including patient age and comorbidities, initial diagnosis and disease status, donor type and transplant modalities (2). More effective approaches for prevention of GVHD (3, 4), fungal infection, and CMV disease (5) have been introduced to prevent and reduce this mortality (6). Several scoring systems, such as the HCT-CI score (7), have been introduced to estimate the risk of transplant-related mortality. In recent years, pretransplant or posttransplant serum ferritin (SF) (8, 9), thought to be a reliable marker of iron overload (IO), has been recognized as another marker of poor outcome after transplantation.

SF levels are frequently elevated in transplant candidates, usually peak in the first few months after transplantation and decrease only very slowly after the transplant (10). This SF peak has been reported previously (11, 12) and we also observed, in our previous study of 70 patients undergoing allo-HSCT, a strong surge of SF values that culminated on day 42 (13). The most common cause for iron overload (IO) during allo-HSCT is the transfusion of numerous RBC units administered before and after transplantation and IO may already be seen after transfusion of only 10-20 units (14). Then, suppression of erythropoiesis after conditioning also contributes to this IO, resulting in decreased marrow demand for iron, saturation of transferrin by non-utilization of SeFe, and further iron deposition in tissues (15). Finally, the cytotoxic action of the conditioning chemotherapy may lead to iron release from dying RBC (16).

Many studies have reported that pretransplant SF predicts for poor outcomes of patients after allo-HSCT (17-27). Indeed, several studies demonstrated that elevated pretransplant SF was associated with inferior OS (17, 19-23, 27-36) and PFS (17, 24, 31, 36-39), as well as a higher risk of NRM (20, 24, 27, 28, 31, 36, 40-42), VOD (22, 23, 43) and infectious events (18, 20, 21, 25). Surprisingly, some studies showed that there was an inverse relationship between raised pretransplant SF and cGVHD but no effect on the incidence of aGVHD (31, 42).

High posttransplant SF could also have a negative prognostic impact (9, 41). Indeed, shortly after allo-HSCT, SF levels are considerably elevated (11, 12). Likewise, after conditioning, Tsat is very high and only corrects itself with recovery of erythropoiesis (44). In this early phase, the pro-oxidant properties of elevated levels of NTBI may exacerbate the toxic effects of the conditioning regimen (45) as well as short and long-term complications, including infections (both fungal and bacterial), VOD and GVHD (46).

However, four studies investigated associations between more specific quantitative markers of IO, such as the liver iron concentration (LIC) measured by MRI and outcomes (47). Among them, two prospective studies by Armand *et al*. and Trottier *et al.* found that LIC was not significantly

associated with OS, relapse, NRM, infections, VOD or GVHD (32, 33). These contrasting results indicate that high pretransplant SF might not be such an accurate marker of IO before allo-HSCT. Indeed, SF does not only correlate with IO, but other factors, such as inflammation, infections, hepatic cytolysis, and renal failure, contribute to increasing its serum levels (48). This suggests that high SF during allo-HSCT is not only related to iron stores but also to several other factors. However, the multifaceted significance of SF and its multiple associations with outcomes have never been addressed comprehensively in the context of allo-HSCT.

Hepcidin, a peptide produced by hepatocytes, is the key regulator of systemic iron balance (49). Hepcidin synthesis is increased by IO and inflammation and decreased in case of iron deficiency, anemia, stimulated erythropoiesis or hypoxia, leading to reduced versus enhanced iron availability for erythropoiesis, respectively (48, 49). Iron regulates hepcidin production through BMP-6 that activates the BMP-SMAD pathway (49, 50). Inflammation-based regulation is mainly dependent on inflammatory cytokines such as IL-6 (49) but the BMP-SMAD pathway is also involved (51, 52). ERFE, which is released by erythroblasts through STAT-5 activation upon erythropoietin stimulation, is the erythroid regulator of iron metabolism, inhibiting hepcidin production (53, 54).

We previously demonstrated significant changes of serum hepcidin levels from pretransplant through day 180 in patients with hematological malignancies after autologous and allogeneic HSCT (13, 55). Our data indicated that hepcidin levels before allo-HSCT were higher than hepcidin levels before auto-HSCT or in normal subjects. Levels then increased significantly over baseline and peaked on day 7, paralleling the kinetics of SF and Tsat and inversely following those of ERFE and sTfR, a quantitative marker of erythropoiesis. This increase could be explained by hepcidin upregulation from inflammation and IO (49) and by downregulation of ERFE production following post-conditioning myelosuppression (56). Moreover, we showed that pretransplant hepcidin was strongly associated with the number of previous RBC transfusions as well as with SF values. Nevertheless, after conditioning, the major determinants of hepcidin production were iron stores and erythropoietic activity, supporting the experimental evidence that suppression of hepcidin during anemia requires erythropoietic activity (55, 57). However, contrarily to other situations, the stimulating effect of IO during allo-HSCT appeared to dominate the inhibitory effect of erythropoietic activity on hepcidin production (13).

Only four studies have explored the effect of pretransplant hepcidin on allo-HSCT outcomes (58-61). Among them, Sakamoto et al (61) were the only ones to report that elevated pretransplant hepcidin levels were associated with poor OS at 3 years, a high incidence of infectious events and decreased platelet engraftment, but not with NRM or disease relapse. However, to our knowledge, no study analyzed the impact of pretransplant serum hepcidin and SF on allo-HSCT outcomes

considering iron parameters, other biological markers, and multiple clinical characteristics in a large cohort of patients.

In this study, we examined the association of an array of biological (including pretransplant SF and hepcidin levels) and clinical factors with OS, PFS, engraftment, toxicities, infections, and acute or chronic GVHD in a large cohort of 502 patients with at least 5 years of follow-up after allo-HSCT. First, we aimed at determining the influence of pretransplant SF and pretransplant hepcidin levels on early and long-term outcomes after allogeneic HCT. Secondly, we examined whether high pretransplant SF is an independent prognostic marker after allogeneic HCT.

## 2.2 Patients and methods

### 2.2.1 Study population

We collected, in a prospective database of transplant patients, biological parameters, pretransplant clinical data and posttransplant clinical outcomes in 502 patients who underwent allogeneic HSCT at the CHU of Liège between 1999 and 2012. Serum samples were also collected and stored prospectively in our biobank before conditioning, weekly thereafter through day 100, and at subsequent visits during follow-up. Patients (or their guardian if of minor age) signed an Ethics Committee-approved informed consent form for the collection of clinical data as well as blood samples for research purposes. Pretransplant patient characteristics are described in **Table 1**. The conditioning regimens were classified as myeloablative (MA) and non-myeloablative (NMA) according to Bacigalupo et al (62). The patients were divided into groups depending on their pretransplant levels of serum hepcidin (4 subgroups) or SF (4 subgroups). The cutoff points for the hepcidin and SF levels were set at their quartile values (Q). Of the 502 patients, all patients had an available pretransplant level hepcidin and 403 (80%) had an available pretransplant SF.

### 2.2.2 Laboratory measurements

Laboratory data were routinely monitored on fresh blood from the days prior to the administration of the conditioning regimen (*pre-conditioning* or *pretransplant* refers to such measurements obtained around day -10 before transplantation) until last follow-up posttransplant. Complete blood counts (Hb, neutrophils, lymphocytes, platelets, CRP, creatinine, liver function tests (total bilirubin and alanine amino transferase (ALAT)), albumin and uric acid, parameters of iron metabolism (SeFe, Tsat and SF) were measured using standard laboratory techniques. The SF peak was defined as the highest value between day 0 and day 100 posttransplant. Serum hepcidin-25 was quantified on biobanked serum samples collected before conditioning, by a liquid chromatography coupled to triple quadrupole mass spectrometry method validated, among others, by the MS-8 lab of our institution (63).

**Table 1**: Pretransplant patient clinical characteristics (N=502)

| | | |
|---|---|---|
| **Age (years) M ± SD** | | 48 ± 17 |
| **Sex** | Male | 313 (62%) |
| | Female | 189 (38%) |
| **Performance status (PS)** | 0 | 90 (18%) |
| | 1 | 325 (65%) |
| | 2 | 87 (17%) |
| **Time between diagnostic and transplantation (days) M ± SD** | | 812 ± 1160 |
| **Disease** | Acute leukemia | 185 (37%) |
| | MDS | 67 (13%) |
| | MPN | 36 (7%) |
| | Lymphoid malignancy | 184 (37%) |
| | Other | 30 (6%) |
| **Disease Risk Index (DRI) [47]** | Low | 91 (18%) |
| | Intermediate | 226 (45%) |
| | High | 153 (30%) |
| | Very high | 23 (5%) |
| | Not applicable | 9 (2%) |
| **Number of treatment lines** | 0 | 52 (10%) |
| | 1 | 170 (34%) |
| | 2 or 3 | 183 (36%) |
| | ≥4 | 97 (20%) |
| **Number of pretransplant RBC transfusions M ± SD** | | 19 ± 22 |
| **Number of pretransplant platelet transfusions M ± SD** | | 16 ± 20 |
| **Comorbidity score (HCT-CI) [7]** | 0 | 243 (48%) |
| | 1-2 | 142 (29%) |
| | ≥3 | 117 (23%) |
| **Transplant number** | First transplant | 344 (69%) |
| | 2nd or 3rd transplant | 158 (31%) |
| **Conditioning** | Myeloablative | 195 (39%) |
| | Non myeloablative | 307 (61%) |
| **ATG** | No | 403 (80%) |
| | Yes | 99 (20%) |
| **GVHD prophylaxis** | CNI ± MMF | 302 (60%) |
| | CNI ± MTX | 83 (17%) |
| | CNI ± other | 117 (23%) |
| **Source of stem cells** | Peripheral blood | 459 (91%) |
| | Bone marrow | 22 (4%) |
| | Cord blood | 21 (4%) |
| **CD34+ cell dose ($10^6$/Kg) M ± SD** | | 5.37 ± 4.16 |
| **Graft manipulations** | | 160 (32%) |
| **Donor age (years) M ± SD** | | 37 ± 15 |
| **Donor relationship and HLA** | Related 10/10 | 174 (35%) |
| | Related HLA-mismatched | 28 (5%) |
| | Unrelated 10/10 | 174 (35%) |
| | Unrelated HLA-mismatched | 126 (25%) |
| **ABO compatibility** | Identical | 278 (56%) |
| | Major mismatch | 102 (20%) |
| | Minor mismatch | 98 (20%) |
| | Major + minor mismatch | 23 (4%) |
| | unknown | 1 (0.2%) |
| **Donor sex match** | F donor > M recipient | 120 (24%) |
| | Other | 382 (76%) |
| **Recipient/donor CMV status** | Recipient - /donor - | 142 (28%) |
| | Recipient + /donor - | 160 (32%) |
| | Recipient - /donor + | 55 (11%) |
| | Recipient + /donor + | 145 (29%) |

*ATG*: anti-thymocyte globulin; *CMV*: cytomegalovirus; *CNI*: calcineurin inhibitor; *F*: female; *GVHD*: graft-versus-host disease; *M*: mean; *M recipient*: male recipient; *MDS*: myelodysplastic syndrome; *MMF*: mycophenolate mofetil; *MPN*: myeloproliferative neoplasm; *MTX*: methotrexate; *PBSC*: peripheral blood stem cells; *RBC*: red blood cells; *SD*: standard deviation.

### 2.2.3 Posttransplant outcomes

Posttransplant outcomes are listed in **Table 2**.

**Table 2:** Posttransplant clinical outcomes in all patients (N=502)

| | | |
|---|---|---|
| **Conditioning-induced toxicities (N; %)** | Cardiac | 32 (6%) |
| | Pulmonary | 27 (5%) |
| | Renal | 37 (7%) |
| | Urinary | 13 (3%) |
| | Neurological | 9 (2%) |
| | Cutaneous | 27 (5%) |
| | Mucosal | 168 (33%) |
| | Gastro-intestinal | 119 (24%) |
| | Hepatic | 94 (19%) |
| | VOD | 22 (4%) |
| **Engraftment: median time (days) to (N; %) *** | Neutrophils ≥ 1 $10^9$/L | 13 (434; 95%) |
| | Platelets ≥ 50 $10^9$/L | 19 (307; 80%) |
| | Reticulocytes ≥ 1 % | 16 (419; 93%) |
| | Hemoglobin ≥ 10 g/dL | 73 (309; 89%) |
| **Number of posttransplant RBC transfusions: M ± SD** | | 19 ± 23 |
| **Number of posttransplant platelet transfusions: M ± SD** | | 19 ± 28 |
| **Transfusion independence: median time (days) to (N; %) **** | RBC | 38 (266; 58%) |
| | Platelets | 15 (250; 61%) |
| **Grade II-IV acute GVHD: median time (days) to (N; %)** | | NR (163; 32%) |
| **Chronic GVHD: median time (months) to (N; %)** | | 11.2 (187; 37%) |
| **Chronic GVHD: NIH score (N; %)** | 0 | 315 (63%) |
| | Mild | 41 (8%) |
| | Moderate | 65 (13%) |
| | Severe | 81 (16%) |
| **Antibiotic prophylaxis (N; %)** | | 142 (28%) |
| **Mycotic prophylaxis (N; %)** | | 500 (99%) |
| **First infection: median time (days) to (N; %) ***** | | 24 (415; 89%) |
| **CMV infection: median time (days) to** | | 33 |
| | Reactivation (N; %) | 171 (34%) |
| | Clinical infection (N; %) | 52 (10%) |
| **Zoster infection: median time (days) to (N; %)** | | NR (60; 12%) |
| **Interstitial pneumonia: median time (days) to (N; %) ******** | | NR (73; 15%) |
| **Second cancer: median time (months) to (N; %)** | | NR (42; 8%) |
| **Overall survival: median duration (months) (N; %)** | | 25 (221; 44%) |
| **Progression-free survival: median duration (months) (N; %)** | | 12 (184; 37%) |
| **Non-relapse mortality: median time (months) to (N; %)** | | NR (141; 28%) |
| **Relapse: median time (months) to (N; %)** | | NR (146; 29%) |

*CMV*: cytomegalovirus; *GVHD*: graft-versus-host disease; *M*: mean; *NR*: not reached; *N*: number; *RBC*: red blood cells; *SD*: standard deviation; *VOD*: veno-occlusive disease.

% Represents the absolute % of all patients, not as a survival function

* Patients never decreasing below these thresholds were excluded from this analysis

** Patients never transfused were excluded from this analysis

*** Patients already infected by day 0 were excluded from this analysis

**** Patients who already had an interstitial pneumonia by day 0 were excluded from this analysis

Conditioning-induced toxicities were graded as previously published (64) and VOD was determined based on the EBMT 2016 diagnostic criteria (65). We also collected data on OS, PFS, NRM, infections, acute and chronic GVHD, as well as the recovery of neutrophils (≥0.5, 1 or 2 x $10^9$/L), reticulocytes (≥0.5, 1 or2%), hemoglobin (≥9 or 10 g/dL or normal (defined as 12 g/dL for women and 13,5 g/dL for men, 10.5 g/dL for children 1-2 years and 11.5 g/dL for children 2-12 years of age)), and platelets (≥25, 50, 100 or 150 x $10^9$/L) without transfusion support. The number of posttransplant RBC units transfused was calculated between day 0 and day 180, and the number of platelet transfusions between day 0 and day 100. We also calculated time to RBC and platelet transfusion independence (and the number of pre and posttransplant transfusions), time to develop a first infectious episode, a CMV infection (reactivation or disease), acute and chronic GVHD, or a second cancer. We graded acute GVHD from I to IV according to the consensus published by Harris *et al* in 2016 (66). Chronic GVHD was diagnosed and graded according to the National Institutes of Health Consensus criteria (67). The competing events in analyses of cumulative incidence were defined as death or second allo-HSCT.

### 2.2.4 Statistical analyses

Results were expressed as mean ± SD unless otherwise stated. Before statistical analyses, some parameters were log-transformed or square root-transformed to normalize their distribution (logarithm for SeFe, Tsat, SF, CRP, creatinine, total bilirubin, ALAT, hepcidin and SF peak; square root for neutrophils, lymphocytes, monocytes, and platelets).

Pretransplant patient characteristics were compared between subgroups (pretransplant hepcidin or SF quartiles (Q)) using the Chi-square test for qualitative variables and Kruskal-Wallis test for quantitative variables.

Univariate and multivariate linear regression models were used to explain hepcidin and SF levels pretransplant and at each time point with respect to covariates. In multivariate analyses, we report $R^2$ to provide a global appreciation of the accuracy of the model, as well as β-coefficients (and standard error (SE)) for significant individual parameters.

Survival (OS and PFS), relapse, engraftment (hemoglobin, reticulocytes, neutrophils, platelets, and transfusion independence), as well as GVHD and infection incidences were estimated using the Kaplan–Meier method. We also compared these outcomes with the log-rank test between groups stratified according to Q of pretransplant serum hepcidin or SF. The Harrel's C statistics (concordance index) was utilized to assess which parameter, among hepcidin and SF, exhibited best goodness of fit for these outcomes. Chi-square tests were used to investigate the difference in frequency of conditioning-related toxicities between the same subgroups. Cumulative incidence of NRM was estimated by standard methods, with relapse and progression treated as competing risks. Groups were compared by the Gray test.

We then investigated biological and clinical parameters associated with these outcomes. All biological values measured before conditioning and on the day of transplantation and all clinical characteristics listed in **Table 1** were considered confounders and were tested as covariates in univariate analyses. All pre-conditioning biological and all clinical variables with a p-value < 0.15 in univariate analysis were included in multivariate analyses using the stepwise method.

Factors influencing conditioning-related toxicities (considered as binary variables) were analyzed using logistic regression and odds ratios (OR) were reported, whereas a Cox proportional-hazard model (HR) was applied to assess factors that potentially influenced survival outcome (OS, PFS and NRM), engraftment, GVHD and infectious complications and hazard ratios (HR) were reported. Linear regression models including pretransplant biological values and clinical parameters were performed to explain the numbers of pretransplant and posttransplant RBC and platelet transfusions (β-coefficients and SE for significant individual parameters are mentioned).

Statistical analyses were performed in SAS version 9.4 (SAS Institute, Cary, NC, USA) and graphs were done using R version 3.6.1. or GraphPad Prism version 9 (GraphPad Software, San Diego, CA). P-values < 0.05 were considered as statistically significant.

## 2.3 Results

### 2.3.1 Significance of pre-conditioning serum ferritin and hepcidin values

Pretransplant clinical characteristics of the 502 patients are described in **Table 1**. Mean age was 48 years (range 2-74 years). Our cohort also included a small pediatric population, consisting of 33 patients. The median pre-conditioning serum hepcidin level was 25.13 ng/mL (range 0.4-345.1) and the median SF level was 1,083 ng/mL (range 6-21,466). The two values were highly correlated with each other ($r=0.61$, $p<0.0001$; **Figure 1**).

SF on day 0 remained strongly correlated with pretransplant SF ($r=0.91$, $p<0.0001$). The median SF peak was 3,419 ng/ml (range 76-168,306) in the first 100 days after transplantation, which was reached after a median of 42 days. This peak correlated significantly with pretransplant hepcidin ($r=0.36$, $p<0.0001$) or SF ($r=0.65$, $p<0.0001$).

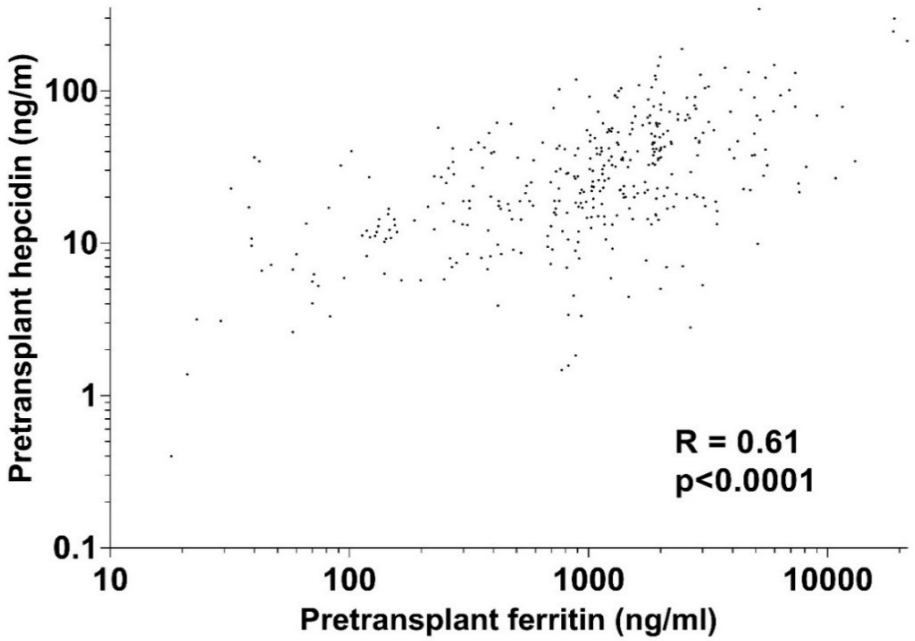


**Figure 1:** Correlations between same day pre-conditioning serum hepcidin and pretransplant serum ferritin in the whole group of patients (n=401; r=0.61, p<0.0001)

Patients were divided into four quartiles according to pre-conditioning serum hepcidin levels, i.e. the Q1-hepcidin group (≤ 12.36 ng/mL; n=126), Q2-hepcidin group (> 12.37 and ≤ 25.13 ng/mL; n=125), Q3-hepcidin group (> 25.13 and ≤ 46.36 ng/mL; n=126) and the Q4-hepcidin group (> 46.36 ng/mL; n=125). PS (p<0.0001), HCT-CI score (p=0.009), number of previous treatment lines (p=0.04), as well as numbers of pretransplant RBC (p<0.0001) and platelet (p<0.0001) transfusions were significantly higher in the Q4-hepcidin group compared to the others. There were also more acute leukemias (AL) (52% vs 21%, p<0.0001), NMA conditionings (62% vs 32%, p=0.03), as well as more HLA-mismatched unrelated (33% vs 24%; p=0.01), but fewer HLA-identical sibling donors, in the Q4-hepcidin compared to the Q1-hepcidin group. There was no significant difference for the other characteristics between the groups based on hepcidin quartiles.

Patients were also divided into four quartiles according to pre-conditioning SF levels, i.e. the Q1-SF group (SF ≤ 390 ng/ml; n=101), the Q2-SF group (SF > 390 and ≤ 1083 ng/ml; n=101), the Q3-SF group (SF > 1083 and ≤ 1933 ng/ml; n=101) and the Q4-SF group (SF > 1933 ng/ml; n=100). The same differences were observed between SF quartiles, except for the HCT-CI score and donor relationships for which no significant difference was observed. In addition, there were more high-risk and fewer low-risk DRI (68) in the Q4-SF quartile compared to the others (p=0.02). There was also a larger use of MMF and a lower use of MTX for GVHD prophylaxis (p=0.002) in the Q1-SF than in the other quartiles. Finally, time between diagnostic and transplantation was significantly higher in the Q1-SF compared to other groups (p=0.0002). Of note, among patients

in the Q1-, Q2-, Q3- and Q4-SF quartiles, 60%, 37%, 35% and 53% were in the respective same quartile for hepcidin.

Pretransplant biological characteristics of the 502 patients divided in groups based on hepcidin and SF quartiles are described in **Table 3.** As expected, pre-conditioning iron status (SF, SeFe and Tsat) was significantly higher in patients with high (Q3 and Q4) than those with low (Q1 and Q2) pre-conditioning hepcidin levels (P<0.0001). Patients in the high hepcidin or SF groups (Q3-Q4) had significantly lower Hb, neutrophils, lymphocytes, platelets and serum albumin, and higher CRP and ALAT levels than those in the low hepcidin or SF groups (Q1-Q2), respectively. No differences were observed for total bilirubin, creatinine, or uric acid values.

Multivariate analyses of clinical and biological parameters associated with pre-conditioning serum hepcidin and SF values, as well as the determinants of the SF peak are displayed in **Table 4.** RBC transfusions, CRP, HCT-CI score, PS and low neutrophil count were found to be independently associated with elevated pretransplant serum hepcidin levels (**Table 4.a**). Pre-conditioning SF levels were positively associated with Tsat, ALAT, creatinine, PS score, pretransplant RBC and platelet transfusions, and negatively with Hb, lymphocytes and a diagnosis of MPN or lymphoid malignancy instead of AL (**Table 4.b**). Pre-conditioning hepcidin, peaks of Tsat, CRP, ALAT and creatinine, as well as pretransplant RBC transfusions and HLA-mismatched unrelated donors were positively associated with high SF peaks, whereas nadirs of platelets, neutrophils and albumin, as well as 2$^{nd}$ or 3$^{rd}$ transplants and minor + major ABO mismatches where negatively correlated with the SF peak (**Table 4.c**).

As bilirubin was found associated with survival outcomes (see below), we also examined the significance of pretransplant bilirubin levels. Mean pretransplant bilirubin was normal (**Table 3**) and only 4% of patients had a pretransplant bilirubin value above normal. In multivariate analyses, pretransplant total bilirubin was positively associated with serum iron, ALAT, as well as a diagnosis of myeloproliferative neoplasm or non-malignant hematological disorders compared to AL (data not shown).

**Table 3**: Pretransplant biological characteristics and ferritin peak (** between days 0 and 100) of patients divided according to quartiles of pretransplant hepcidin or ferritin.

**A:** Quartiles of pretransplant hepcidin: 1st quartile (Q1): ≤ 12.37 ng/ml; 2nd quartile (Q2): > 12.37 and ≤ 25.13 ng/ml; 3rd quartile (Q3): > 25.13 and ≤ 46.36 ng/ml; and 4th quartile (Q4): > 46.36 ng/ml. *Hepcidin normal range: 1.35-42.39 ng/m

| Biological variables | Whole cohort (M±SD) | Values in quartiles of hepcidin (M±SD) | | | | P-value |
|---|---|---|---|---|---|---|
| | | Q1 | Q2 | Q3 | Q4 | |
| **Hepcidin (ng/mL)** | 36.17 ± 38.22 | 6.14 ± 3.42 | 18.4 ± 3.45 | 35.60 ± 6.12 | 84.68 ± 47.29 | - |
| **Serum iron (µmol/L)** | 21.5 ± 11.7 | 17.3 ± 10.4 | 22.1 ± 12.4 | 23.7 ± 11.5 | 23.1 ± 11.5 | <0.0001 |
| **Tsat (%)** | 53 ± 29 | 40 ± 27 | 56 ± 28 | 56 ± 29 | 62 ± 29 | <0.0001 |
| **Ferritin (ng/mL)** | 1,622 ± 2,344 | 588 ± 881 | 1,282 ± 1,293 | 1,727 ± 2,012 | 3,160 ± 3,554 | <0.0001 |
| **Hemoglobin (g/dL)** | 10.5 ± 2.0 | 11.3 ± 1.8 | 10.8 ± 1.9 | 10.3 ± 1.9 | 9.4 ± 1.8 | <0.0001 |
| **Ferritin peak (ng/mL)**** | 5,710 ± 10,935 | 3,304 ± 3,350 | 4,493 ± 4,570 | 7,114 ± 18,270 | 8,225 ± 9,860 | 0.002 |
| **Neutrophils ($10^9$/L)** | 2.93 ± 4.38 | 3.99 ± 7.78 | 2.86 ± 2.24 | 2.54 ± 2.06 | 2.31 ± 2.34 | 0.0002 |
| **Lymphocytes ($10^9$/L)** | 1.10 ± 1.33 | 1.30 ± 1.66 | 1.03 ± 0.72 | 1.14 ± 1.63 | 0.94 ± 1.05 | 0.02 |
| **Platelets ($10^9$/L)** | 144 ± 105 | 166 ± 91 | 156 ± 102 | 137 ± 114 | 118 ± 104 | <0.0001 |
| **CRP (mg/L)** | 18 ± 35 | 9 ± 15 | 9.8 ± 14 | 10.4 ± 17.2 | 42.4 ± 58.2 | <0.0001 |
| **Total bilirubin (mg/dL)** | 0.57 ± 0.60 | 0.56 ± 0.59 | 0.52 ± 0.31 | 0.55 ± 0.32 | 0.65 ± 0.93 | 0.41 |
| **ALAT (U/L)** | 34 ± 41 | 26 ± 22 | 37 ± 48 | 31 ± 33 | 41 ± 52 | 0.02 |
| **Albumin (g/L)** | 38.3 ± 4.9 | 39 ± 4.8 | 38.4 ± 4.1 | 39 ± 4.6 | 36.7 ± 5.6 | 0.001 |
| **Creatinine (mg/dL)** | 0.89 ± 0.38 | 0.86 ± 0.3 | 0.88 ± 0.3 | 0.88 ± 0.33 | 0.95 ± 0.53 | 0.87 |
| **Uric acid (mg/dL)** | 4.7 ± 1.8 | 4.7 ± 1.5 | 4.8 ± 1.8 | 4.7 ± 1.8 | 4.35 ± 1.9 | 0.18 |

**B:** Quartiles of pretransplant ferritin: 1st quartile (Q1): ≤ 390 ng/ml; 2nd quartile (Q2): > 390 and ≤ 1,083 ng/ml; 3rd quartile (Q3): > 1,083 and ≤ 1,933 ng/ml; and 4th quartile (Q4): > 1,933 ng/ml.

| Biological variables | Whole cohort (M±SD) | Values in quartiles of ferritin (M±SD) | | | | P-value |
|---|---|---|---|---|---|---|
| | | Q1 | Q2 | Q3 | Q4 | |
| **Hepcidin (ng/mL)** | 36.17 ± 38.22 | 13.77 ± 12.45 | 24.13 ± 20.76 | 41.51 ± 27.26 | 64.70 ± 58.82 | <0.0001 |
| **Serum iron (µmol/L)** | 21.5 ± 11.7 | 15.0 ± 8. 3 | 20.8 ± 10.3 | 25.1 ± 12.5 | 23.4 ± 11.2 | <0.0001 |
| **Tsat (%)** | 53 ± 29 | 29 ± 18 | 51 ± 26 | 62 ± 28 | 73 ± 25 | <0.0001 |
| **Ferritin (ng/mL)** | 1,622 ± 2.344 | 163 ± 110 | 770 ± 207 | 1,486 ± 279 | 4,254 ± 3,500 | - |
| **Ferritin peak (ng/mL)**** | 5,730 ± 10,935 | 2,188 ± 2,925 | 4,146 ± 4,388 | 7,486 ± 17,26 | 9,260 ± 8,904 | <0.0001 |
| **Hemoglobin (g/dL)** | 10.5 ± 2.0 | 11.9 ± 1.7 | 10.8 ± 1.9 | 10.2 ± 1.7 | 9.0 ± 1.6 | <0.0001 |
| **Neutrophils ($10^9$/L)** | 2.93 ± 4.38 | 4.06 ± 7.08 | 2.68 ± 1.97 | 2.46 ± 1.82 | 2.10 ± 2.44 | 0.0001 |
| **Lymphocytes ($10^9$/L)** | 1.10 ± 1.33 | 1.38 ± 1.76 | 1.20 ± 1.69 | 0.93 ± 1.03 | 0.92 ± 0.80 | <0.0001 |
| **Platelets ($10^9$/L)** | 144 ± 105 | 187 ± 94 | 143 ± 92 | 128 ± 78 | 106 ± 118 | <0.0001 |
| **CRP (mg/L)** | 18 ± 35 | 9 ± 10 | 10 ± 21 | 14 ± 23 | 38 ± 58 | <0.0001 |
| **Total bilirubin (mg/dL)** | 0.57 ± 0.60 | 0.56 ± 0.61 | 0.54 ± 0.39 | 0.55 ± 0.28 | 0.72 ± 1.01 | 0.09 |
| **ALAT (U/L)** | 34 ± 41 | 23 ± 25 | 27 ± 19 | 41 ± 63 | 52 ± 48 | <0.0001 |
| **Albumin (g/L)** | 38.3 ± 4.9 | 39.8 ± 4 | 38.5 ± 4.2 | 38.6 ± 4.5 | 36.6 ± 5.3 | <0.0001 |
| **Creatinine (mg/dL)** | 0.89 ± 0.38 | 0.87 ± 0.31 | 0.87 ± 0.32 | 0.86 ± 0.37 | 0.99 ± 0.50 | 0.15 |
| **Uric acid (mg/dL)** | 4.7 ± 1.8 | 4.8 ± 1.6 | 4.6 ± 1.7 | 4.6 ± 1.9 | 4.6 ± 2.1 | 0.85 |

*ALAT*: alanine aminotransferase; *CRP*: C reactive protein; *Tsat*: transferrin saturation.

**Table 4**: Multiple linear regression of parameters (that had a p-value<0.15 in univariate analyses) influencing pretransplant serum hepcidin ($R^2$=0.44, p<0.0001) and ferritin ($R^2$=0.67, p<0.0001), as well as posttransplant ferritin peak ($R^2$=0.72, p<0.0001). Other parameters were included into the model but were found not significant in multivariate analyses:

**For pretransplant hepcidin (4.a)**: diagnosis, DRI score, pretransplant platelet transfusions, serum iron, hemoglobin, platelets, lymphocytes, total bilirubin, ALAT, creatinine, and uric acid.
**For pretransplant ferritin (4.b)**: age, sex, BMI, number of previous treatment lines, transplant number, DRI score, HTC-CI score, serum iron, platelets, neutrophils, CRP, total bilirubin, and uric acid.
**For ferritin peak (4.c)**: age, BMI, performance status, HCT-CI, DRI score, diagnosis, delay between diagnosis and transplant, number of treatment lines, pretransplant platelet transfusions, conditioning, use of ATG, donor age, graft manipulations, GVHD prophylaxis, antibiotic prophylaxis, CMV status, CD34+ cell dose; serum iron peak, hemoglobin nadir, lymphocyte nadir, peak of total bilirubin and nadir of uric acid before day 100.

| 4.a Multivariate analysis of pretransplant serum hepcidin | β-coefficient | SE | P-value |
|---|---|---|---|
| **Pretransplant serum CRP (mg/L)** | 0.13 | 0.04 | 0.0003 |
| **Pretransplant neutrophils ($10^9$/L)** | -0.14 | 0.06 | 0.01 |
| **Pretransplant RBC transfusions (N)** | 0.18 | 0.02 | <0.0001 |
| **Comorbidity score (HCT-CI)** | 0.09 | 0.03 | 0.0008 |
| **PS score** | 0.24 | 0.08 | 0.02 |

| 4.b Multivariate analysis of pretransplant serum ferritin | β-coefficient | SE | P-value |
|---|---|---|---|
| **Pretransplant transferrin saturation (%)** | 0.47 | 0.08 | <0.0001 |
| **Pretransplant hemoglobin (g/dL)** | -1.25 | 0.28 | <0.0001 |
| **Pretransplant ALAT (U/L)** | 0.23 | 0.06 | 0.0003 |
| **Pretransplant creatinine (mg/dL)** | 0.37 | 0.11 | 0.0006 |
| **Pretransplant lymphocytes ($10^9$/L)** | -0.20 | 0.09 | 0.02 |
| **Pretransplant RBC transfusions (N)** | 0.16 | 0.03 | <0.0001 |
| **Pretransplant platelet transfusions (N)** | 0.06 | 0.03 | 0.046 |
| **Myeloproliferative neoplasm (vs acute leukemia)** | -0.75 | 0.17 | <0.0001 |
| **Lymphoid malignancy (vs acute leukemia)** | -0.44 | 0.11 | <0.0001 |
| **PS score** | 0.23 | 0.08 | 0.006 |

| 4.c Multivariate analysis of posttransplant ferritin peak | β-coefficient | SE | P-value |
|---|---|---|---|
| **Pretransplant serum hepcidin (ng/mL)** | 0.15 | 0.04 | <0.0001 |
| **Transferrin saturation peak (%) before day 100** | 0.59 | 0.12 | <0.0001 |
| **Platelet nadir ($10^9$/L) before day 100** | -0.04 | 0.02 | 0.007 |
| **Neutrophil nadir ($10^9$/L) before day 100** | -0.29 | 0.09 | 0.003 |
| **CRP peak (mg/L) before day 100** | 0.13 | 0.04 | 0.0005 |
| **ALAT peak (U/L) before day 100** | 0.19 | 0.04 | <0.0001 |
| **Creatinine peak (mg/dL) before day 100** | 0.16 | 0.08 | 0.04 |
| **Serum albumin nadir (g/L) before day 100** | -0.04 | 0.01 | <0.0001 |
| **Pretransplant RBC transfusions (N)** | 0.08 | 0.02 | <0.0001 |
| **HLA-mismatched unrelated vs HLA identical sibling donor** | 0.22 | 0.07 | 0.002 |
| **ABO compatibility (minor + major mismatch vs identical)** | -0.46 | 0.15 | 0.003 |
| **2nd or 3rd transplant (vs first transplant)** | -0.19 | 0.07 | 0.01 |

*ALAT*: alanine aminotransferase; *ATG:* anti-thymocyte globulin*; BMI:* body mass index*; CMV*: cytomegalovirus; *CRP*: C reactive protein; *DRI*: disease risk index; *GVHD*: graft-versus-host disease; *HCT-CI:* comorbidity score; *HLA:* human leucocyte antigen*; PS*: performance status; *RBC*: red blood cells; *SE*: standard error.

### 2.3.2 Pre-conditioning serum ferritin and hepcidin as predictors of posttransplant outcomes

Overall posttransplant patient outcomes are shown in **Table 2**. The minimum follow-up duration for living patients was set at 5 years, with a median follow-up time of 9.3 years (range 5-15.8 years).

#### *Overall survival (OS), progression-free survival (PFS), relapse and non-relapse mortality (NRM)*

**OS** was 81% at 100 days, 60% at 1 year and 32% at 10 years (median 25.1 months; **Figure 2A**). The main causes of death within the first 100 days were relapse (n=28, 29% of deaths), bacterial infections (n=14, 14% of deaths) and organ failure (n=10, 10% of deaths). Between days 100 and 365, relapse (n=47, 56% of deaths) was the leading causes of death, while after day 365, relapse (n=31, 45% of deaths) and chronic GVHD (n=12, 17% of deaths) were the most prevalent.

Patients in the Q4-hepcidin group had a significantly lower OS than those in the other groups ($p<0.0001$; **Figure 2B**). Likewise, OS was significantly lower in the Q4-SF group, but also in the Q3-SF group, compared to the others ($p<0.0001$; **Figure 2C**). **PFS** was 75% at 100 days, 50% at 1 year and 26% at 10 years (median 12.1 months; **Figure 2D**). Patients in the Q4-hepcidin group ($P=0.0006$; **Figure 2E**) as well as those in the Q4-SF group ($p=0.0003$; **Figure 2F**) had inferior PFS compared to patients with lower hepcidin or SF values.

In univariate Cox proportional-hazard models, high pre-conditioning serum hepcidin or high pre-conditioning SF were associated with decreased **OS** at day 100 (HR 1.49 and 1.54, $p=0.0003$ and $p<0.0001$, respectively), 1 year (HR 1.30 and 1.43, $p=0.0006$ and $p<0.0001$, respectively) and 10 years (HR 1.24 and 1.25, $p=0.0005$ and $p<0.0001$, respectively). In bivariate analyses with stepwise selection, pre-conditioning SF was a better predictor than pre-conditioning hepcidin for OS at day 100 (HR 1.54, $p<0.0001$ and Harrell's C=0.64), 1 year (HR 2.13, $p<0.0001$ and Harrell's C=0.61) or 10 years (HR 1.25, $p<0.0001$ and Harrell's C=0.61) after transplantation.

In multivariate analyses, pre-conditioning serum hepcidin levels were no longer significantly associated with OS at day 100, 1 year and 10 years, while pretransplant SF was significantly correlated with poor long-term OS (**Table 5**). Other determinants of 100-day OS (**Table 5.a**) were pretransplant total bilirubin, donor type, source of stem cells and fungal prophylaxis. SF on day 0 would also be a significant risk factor of poor OS if we included it in the model (HR 1.65, $p=0.0009$; data not shown). High pretransplant total bilirubin, PS score, donor type, fungal prophylaxis and type of conditioning were significant risk factors for death at 1 year (**Table 5.b**). CRP on day 0 would also be linked to worse OS if we included it into the model (HR 1.36, $p<0.0001$; data not shown). High pretransplant SF and total bilirubin, DRI score, PS score and age were determinants of worse long-term OS (**Table 5.c**). SF on day 0 would also be a risk factor of poor OS if we included it in the model (HR 1.46, $p<0.0001$; data not shown).

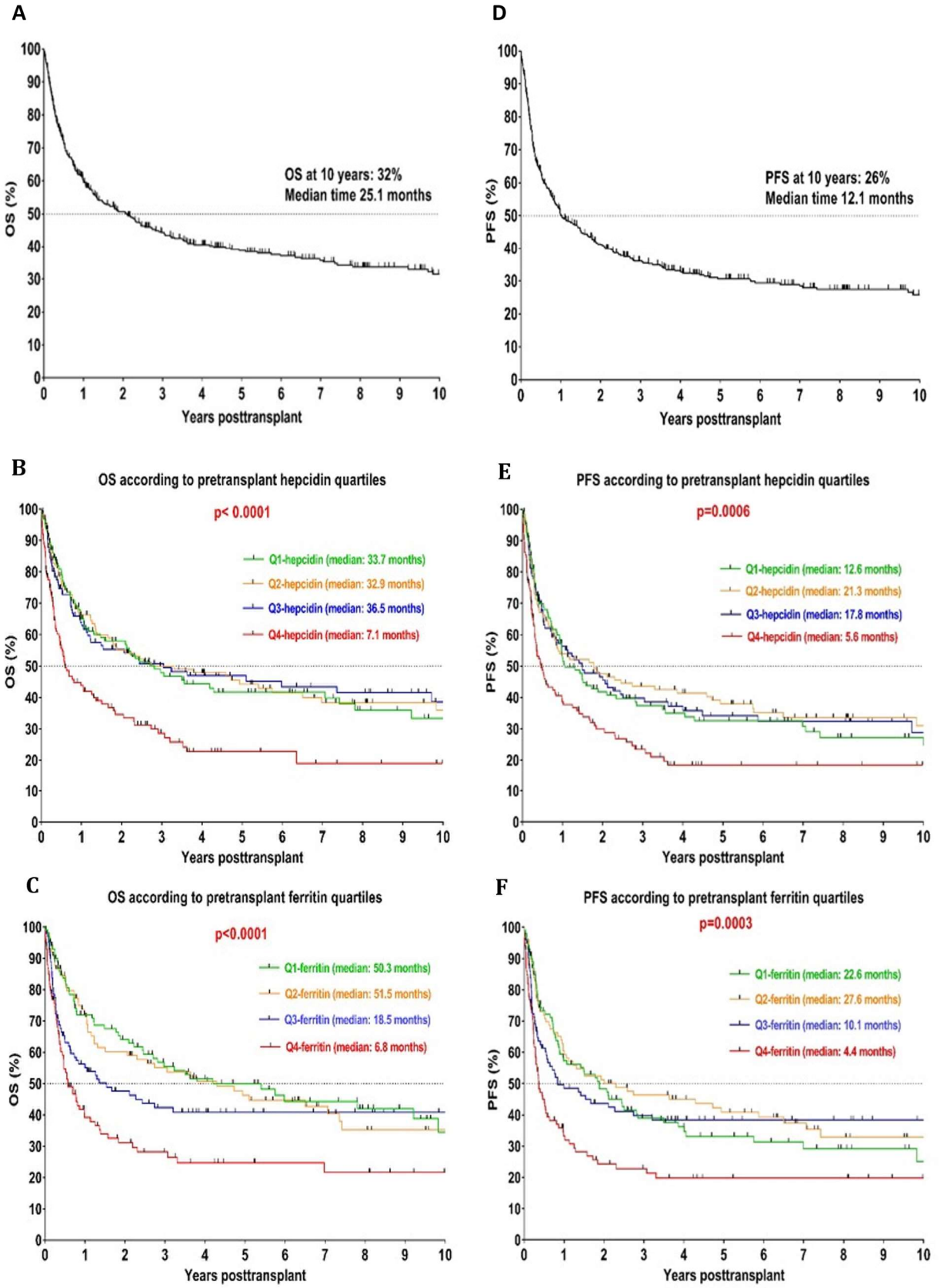


**Figure 2: Survival outcome after allogeneic HSCT:** Long-term overall survival (A, B, C) and long-term PFS (D, E, F) in all patients (A, D) as well as in patients stratified by pretransplant serum hepcidin quartiles (B and E) or pretransplant serum ferritin quartiles (C and F).

**Table 5**: Cox proportional-hazard model of parameters (that had a p-value <0.15 in univariate analyses) impacting OS at 100 days (5.a), 1 year (5.b) and 10 years (5.c) after HCT. Other parameters were included into the model but were found not significant in multivariate analyses:

**For 100-day OS (5.a):** age, BMI, PS, DRI score, number of treatment lines, conditioning, graft manipulations, GVHD prophylaxis; pretransplant RBC and platelet transfusions, hepcidin (p=0.0003), ferritin (p<0.0001), Tsat, CRP, creatinine, Hb, platelets, neutrophils, and lymphocytes.
**For 1-year OS (5.b):** DRI score, diagnosis, number of treatment lines, source of stem cells, graft manipulations, GVHD prophylaxis; pretransplant RBC and platelet transfusions, hepcidin (p=0.0006), ferritin (p<0.0001), serum iron, CRP, creatinine, Hb, platelets, and lymphocytes.
**For 10-year OS (5.c):** number of treatment lines, HCT-CI score, donor type, CMV status, mycotic prophylaxis; pretransplant RBC and platelet transfusions, hepcidin (p=0.0005), CRP, creatinine, Hb, and platelets.

| 5.a Multivariate analysis of 100-day overall survival | | | | |
|---|---|---|---|---|
| **Significant parameters** | **P value** | **HR** | **95% CI** | |
| **Pretransplant total bilirubin** | 0.004 | 1.97 | 1.24 | 3.15 |
| **Bone marrow vs peripheral blood (± bone marrow)** | 0.0009 | 4.16 | 1.79 | 9.67 |
| **HLA-mismatched related vs HLA-identical sibling donor** | 0.001 | 4.76 | 1.89 | 12.00 |
| **Mycotic prophylaxis (other vs fluconazole)** | 0.01 | 2.15 | 1.21 | 3.84 |

| 5.b Multivariate analysis of 1-year overall survival | | | | |
|---|---|---|---|---|
| **Significant parameters** | **P value** | **HR** | **95% CI** | |
| **Pretransplant total bilirubin** | 0.0004 | 1.87 | 1.32 | 2.63 |
| **PS score** | <0.0001 | 1.83 | 1.36 | 2.46 |
| **HLA-identical unrelated vs HLA-identical sibling donor** | 0.0001 | 2.70 | 1.63 | 4.49 |
| **HLA-mismatched related vs HLA-identical sibling donor** | 0.001 | 3.46 | 1.64 | 7.30 |
| **Mycotic prophylaxis (other vs fluconazole)** | 0.02 | 1.58 | 1.09 | 2.29 |
| **Type of conditioning (MA vs NMA)** | 0.03 | 1.55 | 1.05 | 2.28 |

| 5.c Multivariate analysis of 10-year overall survival | | | | |
|---|---|---|---|---|
| **Significant parameters** | **P value** | **HR** | **95% CI** | |
| **Pretransplant serum ferritin** | 0.01 | 1.17 | 1.03 | 1.33 |
| **Pretransplant total bilirubin** | 0.01 | 1.45 | 1.09 | 1.92 |
| **DRI score** | 0.0004 | 1.43 | 1.17 | 1.74 |
| **PS score** | 0.001 | 1.58 | 1.20 | 2.08 |
| **Age** | 0.002 | 1.02 | 1.01 | 1.03 |

*BMI:* body mass index*; CI:* confidence interval*; CMV:* cytomegalovirus*; CRP:* C reactive protein*; DRI:* disease risk index*; HCT-CI:* comorbidity score*; HR:* hazard ratio*; GVHD:* graft-versus-host disease*; HLA:* human leucocyte antigen*; PS:* performance status*; RBC:* red blood cells*; Tsat:* transferrin saturation.

In univariate Cox-proportional-hazard models, high pre-conditioning serum hepcidin or SF were associated with reduced **PFS** on day 100 (HR 1.38 and 1.45, p=0.0008 and p<0.0001, respectively), 1 year (HR 1.16 and 1.26, p=0.03 and p=0.0002, respectively) and 10 years (HR 1.15 and 1.14, p=0.01 and p=0.009, respectively). On bivariate analyses with stepwise selection, pre-conditioning SF was again a better predictor of PFS at day 100 (HR 1.45, p<0.0001 and Harrell's C=0.63), 1 year (HR 1.26, p=0.0002 and Harrell's C=0.60) or 10 years (HR 1.14, p=0.009 and Harrell's C=0.58) after transplantation than pre-conditioning hepcidin. Pre-conditioning hepcidin levels were not found as risk factors in multivariate analyses of PFS (**Table 6**). However, high pre-conditioning SF levels were significantly associated with poor PFS at day 100 (**Table 6.a**) and 1 year (**Table 6.b**) but not in the long-term (**Table 6.c**). Other predictors of PFS are listed in **Table 6**. SF on day 0 would also be a significant risk factor of poor PFS at day 100 and at 1 year if we included it in the model (HR 1.61 and 1.31, p=0.0002 and p=0.0005, respectively; data not shown); likewise, CRP at day 0 would also predict poorer long-term PFS if included into the model (HR 1.14, p=0.02; data not shown).

**The relapse incidence** at 100 days, 1 year, and 10 years was 10%, 30%, and 44%, respectively. Relapse rates were not different between ferritin or hepcidin quartiles. In univariate Cox analysis, neither hepcidin nor ferritin was significantly associated with an increased risk of relapse. The DRI score was the only independent risk factor associated with an increased risk of relapse at 1 year (HR 1.43, p<0.0001). In multivariable analyses, in the long term, the DRI (HR 2.88, p<0.0001), age (HR 1.02, p = 0.006), number of treatment lines (HR 1.11, p=0.03), and total bilirubin (HR 1.44, p=0.046) were significantly associated with more relapses.

However, among the 185 patients with AL (146 acute myeloblastic leukemias and 39 acute lymphoblastic leukemias), median pretransplant hepcidin was 36 ng/mL (63% in Q3-4 hepcidin groups) and SF 1548 ng/mL (73% of AL were in the Q3-4 SF groups). Hepcidin levels did not correlate with relapse. Genetic risk categories were not different between patients below or above this threshold, with 31% vs 41% with normal cytogentics, and 8% vs 11%, 18% vs 10% and 41% vs 36%, respectively with favorable, intermediate or unfavorable cytogenetics. We observed a higher rate of relapse among AL patients with pretransplant SF above compared to those below the median (30% vs 18%, respectively, p=0.005). In this cohort of AL patients, pretransplant SF was significantly associated with relapse in univariate analysis (HR 1.63, p=0.03). However, in multivariate analyses, the only predictive factor for relapse was the DRI score.

**NRM** was 14% at 100 days, 22% at 1 year and 34% at 10 years. Patients in the Q4-hepcidin group (p=0.02) as well as those in the Q3- and Q4-SF groups (p=0.03) had higher NRM compared to patients with lower pre-conditioning hepcidin or SF. In univariate Cox proportional hazard models, elevated pre-conditioning serum hepcidin or SF levels were both associated with increased NRM at day 100 (HR 1.32 and HR 1.47, p=0.048 and p=0.003, respectively), but only

pre-conditioning SF was associated with high NRM at 1 year (HR 1.34, p=0.005) and 10 years (HR 1.21, p=0.02) following transplantation. However, none of them emerged as a significant risk factor in multivariate analyses of NRM (**Table 7**).

We also analyzed survival outcomes across four subgroups based on pretransplant high/low SF (according to the median of 1083 ng/mL) and high/low Tsat (according to the median of 36%). The log-rank test on OS (p<0.0009) and PFS (p=0.004), and the Gray test on NRM (p=0.007) showed significant differences in survival among the subgroups (data not shown). The two subgroups with high SF values ("Low Tsat-High SF" and "High Tsat-High SF") exhibited the worst prognosis in terms of OS, PFS, and NRM, but not for relapse.

**Table 6:** Cox proportional-hazard model of parameters (that had a p-value <0.15 in univariate analyses) impacting PFS at 100 days (6.a), 1 year (6.b) and 10 years (6.c) after HCT. Other parameters were included into the model but were found not significant in multivariate analyses:

**For 100-day PFS (6.a):** DRI, conditioning, GVHD prophylaxis; pretransplant RBC and platelet transfusions, hepcidin (p=0.0008), Tsat, CRP, creatinine, uric acid, Hb, platelets, and neutrophils.
**For 1-year PFS (6.b):** PS, source of stem cells, conditioning, GVHD prophylaxis; pretransplant RBC and platelet transfusions, hepcidin (p=0.03), Tsat, CRP, Hb, platelets, and neutrophils.
**For 10-year PFS (6.c):** HCT-CI, donor type, CMV status; pretransplant RBC and platelet transfusions, hepcidin (p=0.01), ferritin (p=0.009), CRP, creatinine, and Hb.

| 6.a Multivariate analysis of 100-day PFS | | | | |
|---|---|---|---|---|
| **Significant parameters** | **P value** | **HR** | **95% CI** | |
| **Pretransplant serum ferritin** | 0.04 | 1.25 | 1.02 | 1.53 |
| **Pretransplant total bilirubin** | 0.006 | 1.71 | 1.17 | 2.52 |
| **HLA-mismatched related vs HLA-identical sibling donor** | 0.0007 | 4.24 | 1.84 | 9.76 |
| **PS score** | 0.003 | 1.89 | 1.24 | 2.88 |
| **Bone marrow vs peripheral blood (± bone marrow)** | 0.003 | 0.30 | 0.14 | 0.67 |
| **Number of treatment lines** | 0.01 | 1.19 | 1.04 | 1.36 |
| **Mycotic prophylaxis (other vs fluconazole)** | 0.03 | 1.68 | 1.04 | 2.71 |

| 6.b Multivariate analysis of 1-year PFS | | | | |
|---|---|---|---|---|
| **Significant parameters** | **P value** | **HR** | **95% CI** | |
| **Pretransplant serum ferritin** | 0.02 | 1.19 | 1.03 | 1.37 |
| **Pretransplant total bilirubin** | 0.02 | 1.46 | 1.06 | 2.02 |
| **Myelodysplastic syndrome (vs acute leukemia)** | 0.002 | 2.07 | 1.31 | 3.26 |
| **DRI score** | 0.004 | 1.40 | 1.11 | 1.77 |
| **Number of treatment lines** | 0.006 | 1.19 | 1.05 | 1.35 |
| **Sex match (other vs F donor to M recipient)** | 0.007 | 0.56 | 0.37 | 0.85 |
| **Mycotic prophylaxis (other vs fluconazole)** | 0.03 | 1.43 | 1.04 | 1.98 |

| 6.c Multivariate analysis of 10-year PFS | | | | |
|---|---|---|---|---|
| **Significant parameters** | **P value** | **HR** | **95% CI** | |
| **Pretransplant total bilirubin** | 0.002 | 1.51 | 1.16 | 1.95 |
| **DRI score** | <0.0001 | 1.55 | 1.28 | 1.88 |
| **PS score** | 0.001 | 1.48 | 1.17 | 1.87 |
| **Age** | 0.006 | 1.01 | 1.00 | 1.02 |
| **Number of treatment lines** | 0.04 | 1.09 | 1.00 | 1.17 |
| **Mycotic prophylaxis (other vs fluconazole)** | 0.02 | 1.37 | 1.04 | 1.80 |

*CI:* confidence interval*; CMV*: cytomegalovirus; *CRP*: C reactive protein; *DRI*: disease risk index; *F*: female; *HCT-CI*: comorbidity score; *GVHD*: graft-versus-host disease; *HLA*: human leucocyte antigen; *HR*: hazard ratio; *M*: male; *PS*: performance status; *RBC*: red blood cells; *Tsat*: saturation of transferrin.

**Table 7**: Cox proportional-hazard model of parameters (that had a p-value <0.15 in univariate analyses) impacting NRM at 100 days (7.a), 1 year (7.b) and 10 years (7.c) after HCT. Other parameters were included into the model but were found not significant in multivariate analyses:

**For 100-day NRM (7.a):** age, BMI, PS, GVHD prophylaxis, conditioning, donor type, CMV status, CD34+ dose; pretransplant hepcidin (p=0.048), ferritin (p=0.003), serum iron, CRP, creatinine, Hb, platelets, neutrophils, lymphocytes, and albumin.
**For 1-year NRM (7.b):** PS, conditioning, donor type, CMV status, source of stem cells, CD34+ dose, GVHD prophylaxis; pretransplant RBC and platelet transfusions, ferritin (p=0.005), serum iron, Tsat, creatinine and platelets.
**For 10-year NRM (7.c):** PS, GVHD prophylaxis, graft manipulations; pretransplant hepcidin (p=0.13), ferritin (p=0.02), Tsat, CRP, creatinine, total bilirubin, and albumin.

| 7.a Multivariate analysis of 100-day NRM | | | | |
|---|---|---|---|---|
| **Significant parameters** | **P value** | **HR** | **95% CI** | |
| **Pretransplant total bilirubin** | 0.003 | 1.87 | 1.24 | 2.80 |
| **Pretransplant RBC transfusions** | 0.02 | 1.12 | 1.02 | 1.23 |
| **Bone marrow vs peripheral blood (± bone marrow)** | <0.0001 | 4.73 | 2.30 | 9.74 |
| **Mycotic prophylaxis (other vs fluconazole)** | 0.004 | 2.47 | 1.34 | 4.54 |

| 7.b Multivariate analysis of 1-year NRM | | | | |
|---|---|---|---|---|
| **Significant parameters** | **P value** | **HR** | **95% CI** | |
| **Pretransplant total bilirubin** | 0.0003 | 1.91 | 1.34 | 2.72 |
| **Pretransplant albumin** | 0.01 | 0.93 | 0.89 | 0.98 |
| **Mycotic prophylaxis (other vs fluconazole)** | 0.006 | 1.96 | 1.22 | 3.16 |

| 7.c Multivariate analysis of 10-year NRM | | | | |
|---|---|---|---|---|
| **Significant parameters** | **P value** | **HR** | **95% CI** | |
| **Pretransplant RBC transfusions** | 0.007 | 1.11 | 1.03 | 1.20 |
| **Type of conditioning (NMA vs MA)** | 0.03 | 0.65 | 0.45 | 0.95 |
| **HLA-mismatched unrelated vs HLA-identical sibling donor** | 0.02 | 1.70 | 1.08 | 2.68 |
| **Mycotic prophylaxis (other vs fluconazole)** | 0.003 | 1.77 | 1.22 | 2.56 |

*CI:* confidence interval*; CMV*: cytomegalovirus; *CRP*: C reactive protein; *HCT-CI*: comorbidity score; *GVHD*: graft-versus-host disease; *HLA*: human leucocyte antigen; *HR*: hazard ratio; *MA*: myeloablative conditioning; *NMA*: nonmyeloablative conditioning, *PS*: performance status; *RBC*: red blood cells; *Tsat*: transferrin saturation.

*Conditioning-induced toxicities (64)*

The **rate of each organ toxicity** is shown in **Table 2** and in **Figure 3**. Among the 502 patients, the most frequent toxicities were mucosal (33%), gastrointestinal (24%) and hepatic (19%) toxicities (**Figure 3A**). There was significantly more cutaneous toxicity in the Q3/Q4-hepcidin compared to the Q1-hepcidin quartiles (28% vs 17%, respectively; p=0.007), while there was no significant difference in the rates of other toxicities between hepcidin quartiles (**Figure 3B**). Similarly, in the Q3/Q4-SF compared to Q1-SF quartile, there was more gastro-intestinal (33% vs 10%; p=0.0002), mucosal (42% vs 19%; p=0.0006) and renal toxicities (12 % vs 2 %, respectively; p=0.04), but there was no difference for the other toxicities (**Figure 3C**). High pre-conditioning serum hepcidin was associated with a high risk of cardiac (OR 1.53, p=0.03), renal (OR 1.69, p=0.005) and cutaneous (OR 2.04, p=0.002) toxicities in univariate but not in multivariate models. High pre-conditioning SF was associated with a high risk of cardiac (OR 1.69, p=0.004), renal (OR 1.67, p=0.004), mucosal (OR 1.44, p<0.0001) and gastrointestinal (OR 1.64, p<0.0001) toxicities or VOD (OR 1.54, p=0.047) in univariate but not in multivariate models, except for gastrointestinal toxicity (OR 1.57, p=0.003). On bivariate analyses with stepwise selection, pre-conditioning SF was more predictive than pre-conditioning hepcidin for all (except cutaneous) toxicities.

On multivariate analyses, performance status (OR 3.52, p=0.003) and MA conditioning (OR 16.67, p=0.0002) were identified as the two risk factors for developing **cardiac toxicity**. MA conditioning was the only risk factor explaining **lung toxicity** (OR 8.33, p=0.0002). High pretransplant creatinine (OR 7.18, p=0.002), CRP (OR 1.53, p=0.02) and performance status (OR 2.27, p=0.046), as well as the use of methotrexate (MTX) in the immunosuppressive regimen (OR 23.81, p<0.0001), were risk factors for **kidney toxicity**. **Cutaneous toxicity** was associated with high pre-conditioning hepcidin (OR 1.95, p=0.02) and MA conditioning (OR 33.33, p=0.001). Low pre-conditioning uric acid (OR 1.23, p=0.04), MA conditioning (OR 16.39, p<0.0001), low CD34 cell dose in the graft (OR 1.79, p=0.04) and use of MTX (OR 21.28, p<0.0001) were risk factors for **mucosal toxicity**. **Gastrointestinal toxicity** was more prevalent with high pre-conditioning SF (OR 1.46, p=0.007), low pre-conditioning uric acid (OR 1.49, p<0.0001), and use of MTX (OR 14.9, p<0.0001) or prophylactic quinolones (OR 3.34, p=0.0006). Finally, **liver toxicity** was more likely with older age (OR 1.03, p=0.003), treatment lines before transplant (OR 0.67, p=0.0004), a long delay between diagnosis and transplantation (OR 1.40, p=0.02), high pre-conditioning total bilirubin (OR 2.13, p=0.004), and MA conditioning (OR 10.31, p<0.0001). No variable appeared to be predictive in our multivariate model for **VOD**.

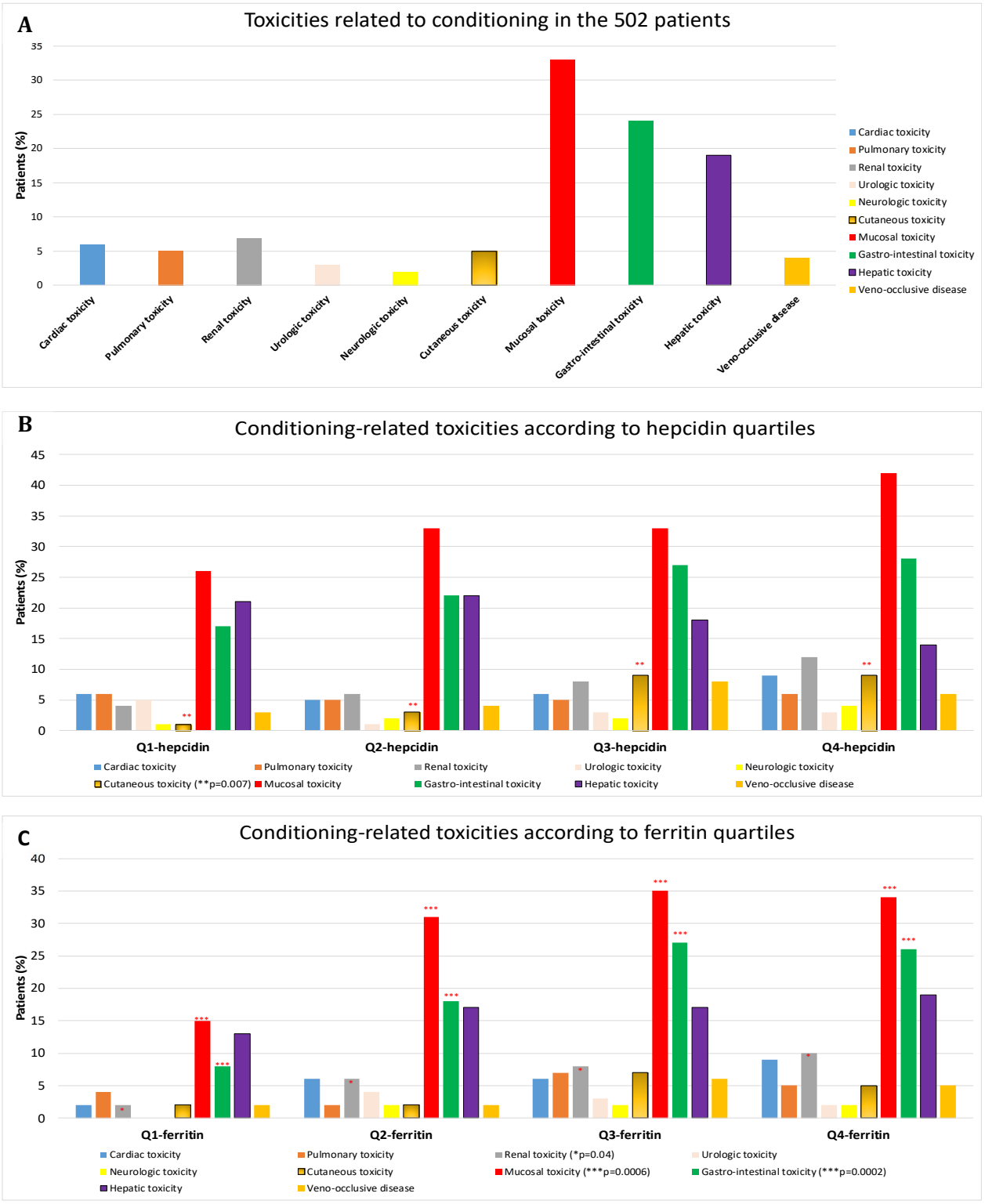


**Figure 3:** Conditioning-related toxicities after allogeneic HSCT in all patients (A), according to serum hepcidin quartiles (B) or serum ferritin quartiles (C).

*Pre and posttransplant transfusions*

Large numbers of **pretransplant RBC and platelet transfusions** were associated with high pre-conditioning serum hepcidin (β-coefficient=1.15, SE=0.09, p<0.0001, and β-coefficient=0.87, SE=0.09, p<0.0001, respectively) and SF (β-coefficient=1.19, SE=0.07, p<0.0001, and β-coefficient=1.00, SE=0.07, p<0.0001, respectively) in univariate but not multivariate analyses. On bivariate analyses with stepwise selection, pre-conditioning SF outperformed hepcidin to predict the number of pretransplant RBC transfusions (β-coefficient=0.59, SE=0.07, p<0.0001 for SF, and β-coefficient=0.36, SE=0.09, p=0.0001 for hepcidin) whereas both contributed to prediction of platelet transfusion rates. In multivariate analyses, diagnosis (MPN had more pretransplant RBC transfusions than AL) and PS were significantly associated with more pretransplant RBC transfusions, whereas age, lymphoproliferative diseases (vs AL) and HCT-CI score were associated with fewer pretransplant RBC transfusions ($R^2$=0.29, p-values between 0.03 and <0.0001). The same parameters (but also the number of pretransplant RBC transfusions) were found to be significantly correlated with the number of pretransplant platelet transfusions ($R^2$=0.36, p-values between 0.02 and <0.0001).

Regarding **posttransplant transfusions**, high pre-conditioning SF levels were positively associated with higher rates of RBC (β-coefficient=0.41, SE=0.09, p<0.0001) and platelet (β-coefficient=0.61, SE=0.08, p<0.0001) transfusions in univariate analysis, while hepcidin was not. In multivariate analyses, high pre-conditioning SF, low Hb, MA conditioning, the use of ATG, a diagnosis of MPN, and a poor PS score were associated with increased posttransplant RBC transfusion needs (**Table 8.a**). Numbers of pretransplant platelet transfusions, pre-conditioning platelet counts, MA conditioning, minor ABO incompatibility, a diagnosis of MPN, and the use of MTX for GVHD prophylaxis, but not SF, were identified as factors associated with a higher number of posttransplant platelet transfusions (**Table 8.b**).

After transplantation, among the 458 (91%) patients transfused with RBC and 407 (81%) with platelets, times to RBC (70% at 1 year, median 38 days) and platelet (80% at 1 year, median 15 days) transfusion independence were longer in the Q4-SF group (p=0.008 and p=0.03, respectively) but did not differ among hepcidin quartiles (**Figures 4A-4F**). In univariate analyses, high pre-conditioning SF, but not hepcidin, was associated with delayed RBC transfusion independence (HR 0.90, p=0.03) and both were correlated with delayed platelet transfusion independence (HR 0.87, p=0.007 for SF, and HR 0.88, p=0.04 for hepcidin). In multivariate analyses, high pre-conditioning CRP, major ABO incompatibility, poor PS and low CD34+ cell doses were correlated with slower RBC transfusion independence (**Table 9.a**). CD34+ cell dose and type of GVH prophylaxis were associated with platelet transfusion independence, whereas poor PS predicted for delayed independence (**Table 9.b**).

**Table 8**: Multiple linear regression of parameters (that had a p-value <0.15 in univariate analyses) impacting the number of posttransplant RBC (8.a, $R^2$=0.62, p<0.0001) and platelet (8.b, $R^2$=0.67, p<0.0001) transfusions. Other parameters were included into the model but were found not significant in multivariate analyses:

**For posttransplant RBC transfusions (8.a)**: age, number of treatment lines, delay between diagnosis and transplant, HCT-CI score, previous transplant, ABO compatibility, source of stem cells, graft manipulations, CD34+ cell dose, GVHD prophylaxis, antibiotic prophylaxis, mycotic prophylaxis; pretransplant RBC or platelet transfusions, Tsat, serum iron, CRP, uric acid, total bilirubin, albumin, platelets, neutrophils, and lymphocytes.

**For posttransplant platelet transfusions (8.b)**: age, BMI, PS score, DRI score, number of treatment lines, delay between diagnosis and transplant, HCT-CI score, previous transplant, source of stem cells, graft manipulations, CD34+ cell dose, donor age, antibiotic prophylaxis, mycotic prophylaxis, use of ATG; pretransplant RBC transfusions, ferritin (p<0.0001), hepcidin (p=0.14), serum iron, Tsat, CRP, creatinine, uric acid, total bilirubin, ALAT, albumin, Hb, neutrophils and lymphocytes.

| 8.a Multivariate analysis of posttransplant RBC transfusions | | | |
|---|---|---|---|
| **Significant parameters** | **β-coefficient** | **SE** | **P-value** |
| **Pretransplant serum ferritin** | 0.28 | 0.51 | 0.002 |
| **Pretransplant hemoglobin** | -2.27 | 0.83 | 0.008 |
| **Myeloproliferative neoplasm (vs acute leukemia)** | 2.30 | 0.51 | <0.0001 |
| **Type of conditioning (NMA vs MA)** | -0.005 | 0.01 | <0.0001 |
| **Use of ATG** | 0.72 | 0.28 | 0.01 |
| **PS score** | 0.57 | 0.24 | 0.02 |

| 8.b Multivariate analysis of posttransplant platelet transfusions | | | |
|---|---|---|---|
| **Significant parameters** | **β-coefficient** | **SE** | **P-value** |
| **Pretransplant platelets** | -0.10 | 0.03 | 0.0009 |
| **Pretransplant platelet transfusions** | 0.20 | 0.05 | 0.0003 |
| **Myeloproliferative neoplasm (vs acute leukemia)** | 1.03 | 0.51 | 0.04 |
| **Type of conditioning (NMA vs MA)** | -0.01 | 0.01 | <0.0001 |
| **ABO compatibility (minor mismatch vs identical)** | 1.02 | 0.32 | 0.002 |
| **GVHD prophylaxis (CNI ± other vs CNI ± MTX)** | -0.75 | 0.32 | 0.02 |

*ATG:* anti-thymocyte globulin*; BMI:* body mass index*; CMV:* cytomegalovirus*; CRP:* C reactive protein*; DRI:* disease risk index*; GVHD:* graft-versus-host disease*; HCT-CI:* comorbidity score*; HLA:* human leucocyte antigen*; MA:* myeloablative*; NMA:* nonmyeloablative*; MNP:* myeloproliferative neoplasm*; PS:* performance status*; RBC:* red blood cells*; SE:* standard error*; Tsat:* transferrin saturation.

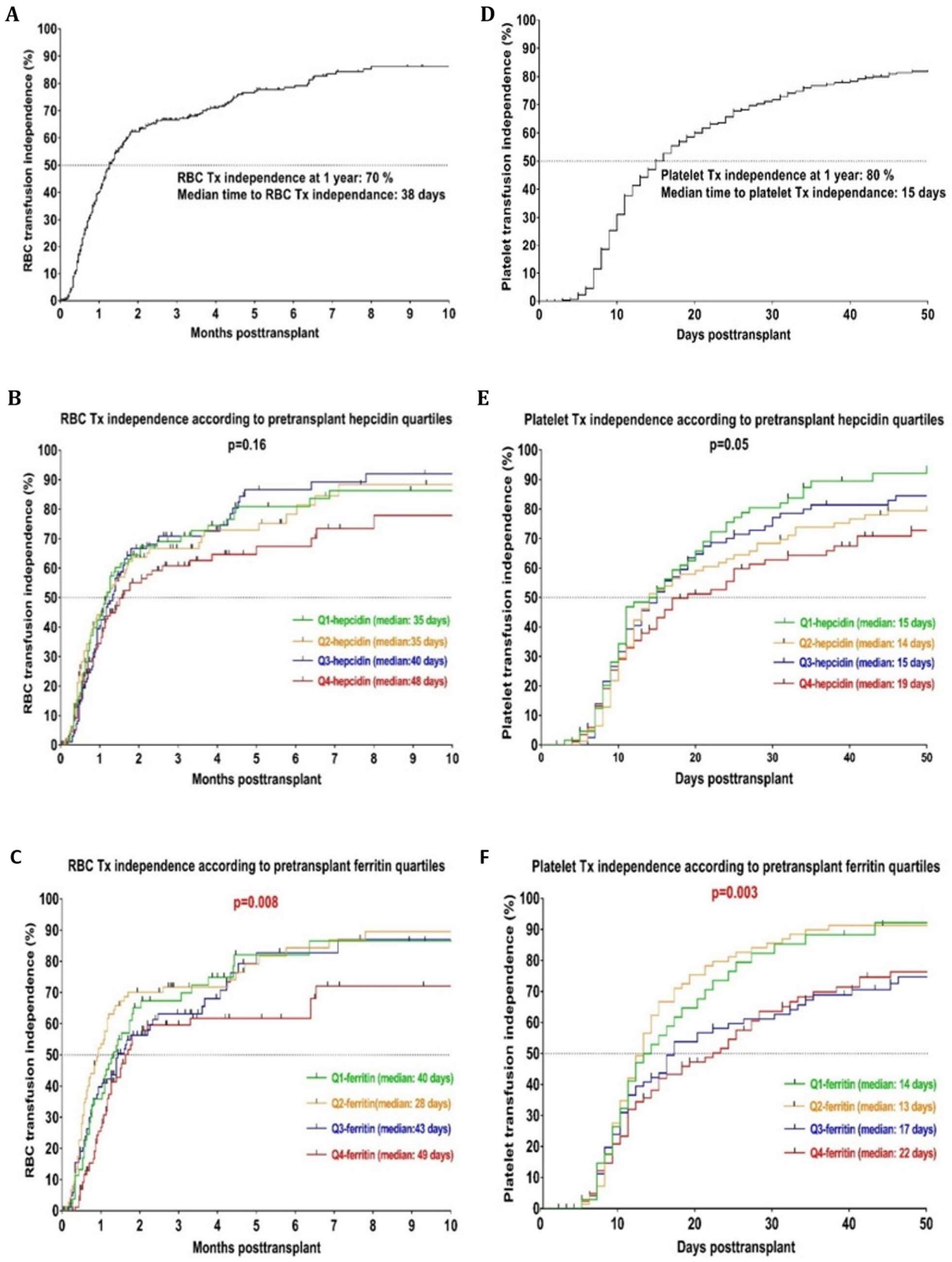


**Figure 4: Time to RBC (A, B, C) or platelet (D, E, F) transfusion independence after allogeneic HSCT** in all patients (A and D) as well as in patients stratified by pretransplant serum hepcidin quartiles (B and E) or pretransplant serum ferritin quartiles (C and F).

**Table 9**: Cox proportional-hazard model of parameters (that had a p-value <0.15 in univariate analyses) impacting time to posttransplant RBC (9.a) and platelet (9.b) transfusion independence. Other parameters were included into the model but were found not significant in multivariate analyses:

**For time to posttransplant RBC transfusion independence (9.a)**: diagnosis, DRI score, donor sex match, source of stem cells, conditioning, GVHD prophylaxis, CMV status, CD34+ cell dose; pretransplant RBC transfusions, hepcidin (p=0.061), ferritin (p=0.03), and neutrophils.
**For time to posttransplant platelet transfusion independence (9.b)**: age, donor type and age, source of stem cells, transplant number, conditioning, use of ATG, mycotic prophylaxis; pretransplant RBC and platelet transfusions, hepcidin (p=0.04), ferritin (p=0.007), CRP, and Hb.

| 9.a Multivariate analysis of time to RBC transfusion independence | | | | |
|---|---|---|---|---|
| **Significant parameters** | **P value** | **HR** | **95% CI** | |
| **Pretransplant CRP** | 0.04 | 0.88 | 0.78 | 0.99 |
| **PS score** | 0.01 | 0.70 | 0.53 | 0.93 |
| **CD34+ cell dose** | 0.03 | 1.25 | 1.02 | 1.52 |
| **Major ABO mismatch vs ABO identical** | 0.001 | 0.55 | 0.38 | 0.79 |

| 9.b Multivariate analysis of time to platelet transfusion independence | | | | |
|---|---|---|---|---|
| **Significant parameters** | **P value** | **HR** | **95% CI** | |
| **PS score** | 0.0003 | 0.64 | 0.50 | 0.82 |
| **CD34+ cell dose** | <0.0001 | 2.35 | 1.60 | 3.45 |
| **GVHD prophylaxis (CNI ± MMF vs CNI ± MTX)** | <0.0001 | 1.74 | 1.40 | 2.17 |
| **GVHD prophylaxis (CNI ± other vs CNI ± MTX)** | 0.0004 | 2.02 | 1.37 | 2.97 |

*CI:* confidence interval*; CRP:* C-reactive protein*; CNI:* calcineurin inhibitor*; CMV:* cytomegalovirus*; DRI:* disease risk index*; GVHD:* graft-versus-host disease; *HR*: hazard ratio; *MTX:* methotrexate*; MMF*: mycophenolate mofetil*; PS:* performance status*; RBC:* red blood cells.

### *Engraftment*

**Times to engraftment** were analyzed after excluding patients who never decreased counts below the thresholds (47 (9%) patients for neutrophil recovery to ≥ 1 $10^9$/L, 117 (23%) for platelet recovery to 50 $10^9$/L, and 43 (8%) for Hb 10 gr/dL). Neutrophil recovery was 96% by day 28 (median 13 days) (**Figure 5A**) and this was significantly slower with higher pre-conditioning SF (**Figure 5C**) but not hepcidin (**Figure 5B**) quartiles. Platelet recovery was 80% by day 50 (median 19 days) (**Figure 5D**) and this was significantly different according to pre-conditioning ferritin (**Figure 5F**) or hepcidin (**Figure 5E**) quartiles. Hemoglobin recovery was 89% at 1 year (median 73 days) (**Figure 5G**) and this was significantly slower with higher SF (**Figure 5I**) but not hepcidin (**Figure 5H**) quartiles.

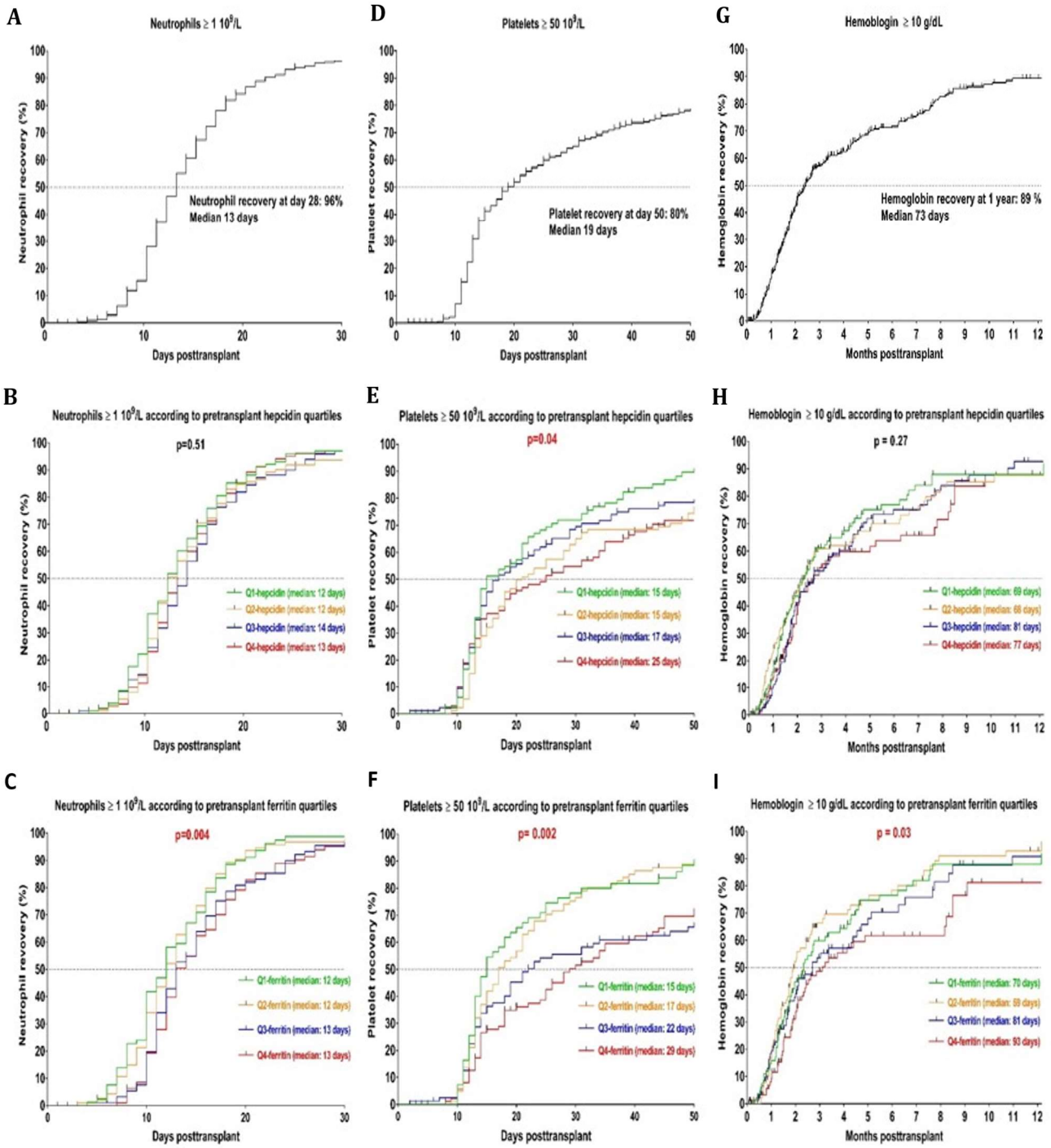


**Figure 5:** Time to neutrophils ≥ 1 $10^9$/L (A, B, C) or platelets 50 ≥ $10^9$/L (D, E, F) or hemoglobin ≥ 10 g/dL (G, H, I) after allogeneic HSCT in all patients (A, D, I) as well as in patients stratified by pretransplant serum hepcidin quartiles (B, E, H) or pretransplant serum ferritin quartiles (C, F, I).

In univariate Cox proportional-hazard models, high pre-conditioning SF, but not hepcidin, was associated with delayed neutrophil recovery (HR 0.89, p=0.003) and both variables were correlated with platelet recovery (HR 0.84, p=0.0003 for SF, and HR 0.90, p=0.04 for hepcidin). Hb recovery to 10 g/dL did not correlate with pre-conditioning SF but with hepcidin (HR 0.90, p=0.04). Moreover, hematocrit (Hct) recovery to 30% was delayed in patients with higher pre-conditioning hepcidin, while recoveries to Hb 9 gr/dL or Hct 27% or 0.5-1-2% reticulocytes were all delayed in the presence of higher pre-conditioning SF values (data not shown).

In multivariate analyses (**Table 10**): neutrophil recovery was delayed with cord blood, major ABO incompatibility and use of ATG, while it was faster when using MMF or other immunosuppressive (IS) drugs (vs MTX) (**Table 10.a**). Platelet recovery was significantly delayed with high pre-conditioning CRP, poor PS, minor ABO incompatibility, graft manipulations and use of ATG, whereas CD34 cell dose and use of MMF or other IS (vs MTX) were associated with faster platelet recovery (**Table 10.b**). Hb recovery was delayed in cases of major ABO mismatch, recipient positive CMV status and poor PS. Conversely, earlier Hb recovery was observed with the use of MMF or other IS (vs MTX) and a higher $CD34^+$ cell dose (**Table 10.c**). CRP level on day 0 would also be a significant risk factor of delayed engraftment of neutrophils, platelets, and Hb if we included it in the model (data not shown).

**Table 10**: Cox proportional-hazard model of parameters (that had a p-value <0.15 in univariate analyses) impacting time to 1.0 $10^9$/L neutrophils (9.a), 50 $10^9$/L platelets (9.b) and 10 g/dL hemoglobin (9.c). Other parameters were included into the model but were found not significant in multivariate analyses:

**For neutrophils (10.a):** age, diagnosis, number of treatment lines, previous transplant, donor type and donor age, donor sex match, CD34+ cell dose, conditioning, antibiotic prophylaxis; pretransplant RBC and platelet transfusions, ferritin (p=0.003), Tsat, total bilirubin, creatinine, uric acid, and Hb.
**For platelets (10.b):** age, diagnosis, DRI, number of treatment lines, delay between diagnosis and transplant, HCT-CI score, source of stem cells, transplant number, donor age, conditioning, antibiotic prophylaxis, mycotic prophylaxis; pretransplant RBC and platelet transfusions, hepcidin (p=0.04), ferritin (p=0.0003), serum iron, Tsat, total bilirubin, uric acid, Hb, platelets, and neutrophils.
**For Hb (10.c):** BMI, diagnosis, DRI score, transplant number, conditioning, use of ATG, antibiotic prophylaxis, mycotic prophylaxis; pretransplant RBC transfusions, hepcidin (p=0.04), serum iron, Tsat, CRP, creatinine, hemoglobin, and lymphocytes.

| 10.a Multivariate analysis of time to 1.0 $10^9$/L neutrophils | | | | |
|---|---|---|---|---|
| **Significant parameters** | **P value** | **HR** | **95% CI** | |
| **Cord blood (± bone marrow/PBSC) vs PBSC (± bone marrow)** | <0.0001 | 0.21 | 0.12 | 0.37 |
| **Use of ATG** | 0.04 | 0.73 | 0.54 | 0.98 |
| **GVHD prophylaxis (CNI ± MMF vs CNI ± MTX)** | 0.002 | 1.73 | 1.22 | 2.44 |
| **GVHD prophylaxis (CNI ± other vs CNI ± MTX)** | 0.0002 | 2.09 | 1.42 | 3.08 |
| **ABO compatibility (major mismatch vs identical)** | 0.009 | 0.68 | 0.51 | 0.91 |

| 10.b Multivariate analysis of time to 50 $10^9$/L platelets | | | | |
|---|---|---|---|---|
| **Significant parameters** | **P value** | **HR** | **95% CI** | |
| **Pretransplant CRP** | 0.001 | 0.84 | 0.75 | 0.93 |
| **PS score** | 0.0002 | 0.62 | 0.48 | 0.80 |
| **CD34+ cell dose** | <0.0001 | 2.05 | 1.63 | 2.54 |
| **Graft manipulations** | 0.009 | 0.64 | 0.46 | 0.90 |
| **Use of ATG** | 0.045 | 0.72 | 0.52 | 0.99 |
| **GVHD prophylaxis (CNI ± MMF vs CNI ± MTX)** | <0.0001 | 3.41 | 2.31 | 5.05 |
| **GVHD prophylaxis (CNI ± other vs CNI ± MTX)** | 0.0005 | 2.20 | 1.41 | 3.42 |
| **ABO compatibility (minor mismatch vs identical)** | 0.006 | 0.59 | 0.41 | 0.86 |

| 10.c Multivariate analysis of time to 10 g/dL hemoglobin | | | | |
|---|---|---|---|---|
| **Significant parameters** | **P value** | **HR** | **95% CI** | |
| **PS score** | 0.008 | 0.72 | 0.57 | 0.92 |
| **CD34+ cell dose** | 0.02 | 1.30 | 1.04 | 1.63 |
| **CMV: Don neg & Rec + vs Don & Rec neg** | 0.0005 | 0.55 | 0.39 | 0.77 |
| **CMV : Don & Rec + vs Don & Rec neg** | <0.0001 | 0.46 | 0.32 | 0.66 |
| **GVHD prophylaxis (CNI ± MMF vs CNI ± MTX)** | <0.0001 | 2.48 | 1.61 | 3.84 |
| **GVHD prophylaxis (CNI ± other vs CNI ± MTX)** | <0.0001 | 2.80 | 1.70 | 4.59 |
| **ABO compatibility (major mismatch vs identical)** | <0.0001 | 0.49 | 0.34 | 0.69 |

*ATG:* anti-thymocyte globulin*; BMI:* body mass index*; CI:* confidence interval*; CMV:* cytomegalovirus*; CNI:* calcineurin inhibitor*; CRP:* C reactive protein*; DRI:* disease risk index*; Don:* donor*; GVHD:* graft-versus-host disease*; HCT-CI:* comorbidity score*; HR:* hazard ratio*; MMF:* mycophenolate mofetil*; MTX:* methotrexate*; PBSC:* peripheral blood stem cells*; PS:* performance status*; RBC:* red blood cells; *Rec*: recipient*; Tsat:* transferrin saturation.

*Infections*

About 83% of patients developed at least **a first infectious episode** by 1 year, most of them in the first 2 months after transplantation (**Figure 6A**); 35 (7%) patients who had a first infectious event by day 0 were excluded from these analyses. While pre-conditioning hepcidin had no discernable effect (**Figure 6B**), patients in the Q4-SF quartile had more infections than those with lower SF values and particularly those in the Q1-SF quartile (p=0.002; **Figure 6C**). Pre-conditioning SF, but not hepcidin, was associated with a higher incidence of a first infectious event in univariate analysis (HR 1.17, p=0.0003). In multivariate analysis, high Tsat, female sex and the use of an unrelated donor were associated with a higher risk of infection. Conversely, NMA conditioning and quinolone prophylaxis were identified as protective factors (**Table 11.a**). SF level on day 0 would also be a significant risk factor for infections if we included it in the model (HR 1.16, p=0.04; data not shown).

About 54 % of patients developed a **first sepsis** by 1 year, most of them in the first months after transplantation (**Figure 6D**); 5 (1%) patients who had sepsis by day 0 were excluded. While pre-conditioning hepcidin had no discernable effect (**Figure 6E**), patients in the Q4-SF quartile had more sepsis than those with lower SF values and particularly those in the Q1-SF quartile (p=0.02; **Figure 6F**). However, neither pre-conditioning SF nor hepcidin correlated with the incidence of sepsis in univariate Cox models. In multivariate analyses, high pre-conditioning creatinine, a mismatched unrelated donor and graft manipulations increased, while NMA conditioning decreased, the risk of sepsis (**Table 11.b**).

The cumulative incidence of **CMV reactivation/infection** was 48% at 1 year, and that of zoster infection was 34% at 10 years. There was no significant correlation between pre-conditioning hepcidin or SF levels and these outcomes. The significant variables in multivariate analyses are presented in **Table 11.c-d.**

Lastly, 13% of patients developed **interstitial pneumonia** (IP) within the first year, with the majority occurring in the initial 2 months after transplantation (Figure 7A). Five (1%) patients who had IP by day 0 were excluded. Pre-conditioning hepcidin did not predict IP, while patients in the Q4-SF quartile experienced IP more frequently than those with lower SF values (p=0.01; **Figure 7B & 7C**). High pre-conditioning SF, but not hepcidin, was associated with a higher risk of IP at 1 year (HR 1.29, p=0.004) in univariate analysis but not in multivariate analysis. Multivariate analyses identified several other predictors of IP (**Table 11.e**).

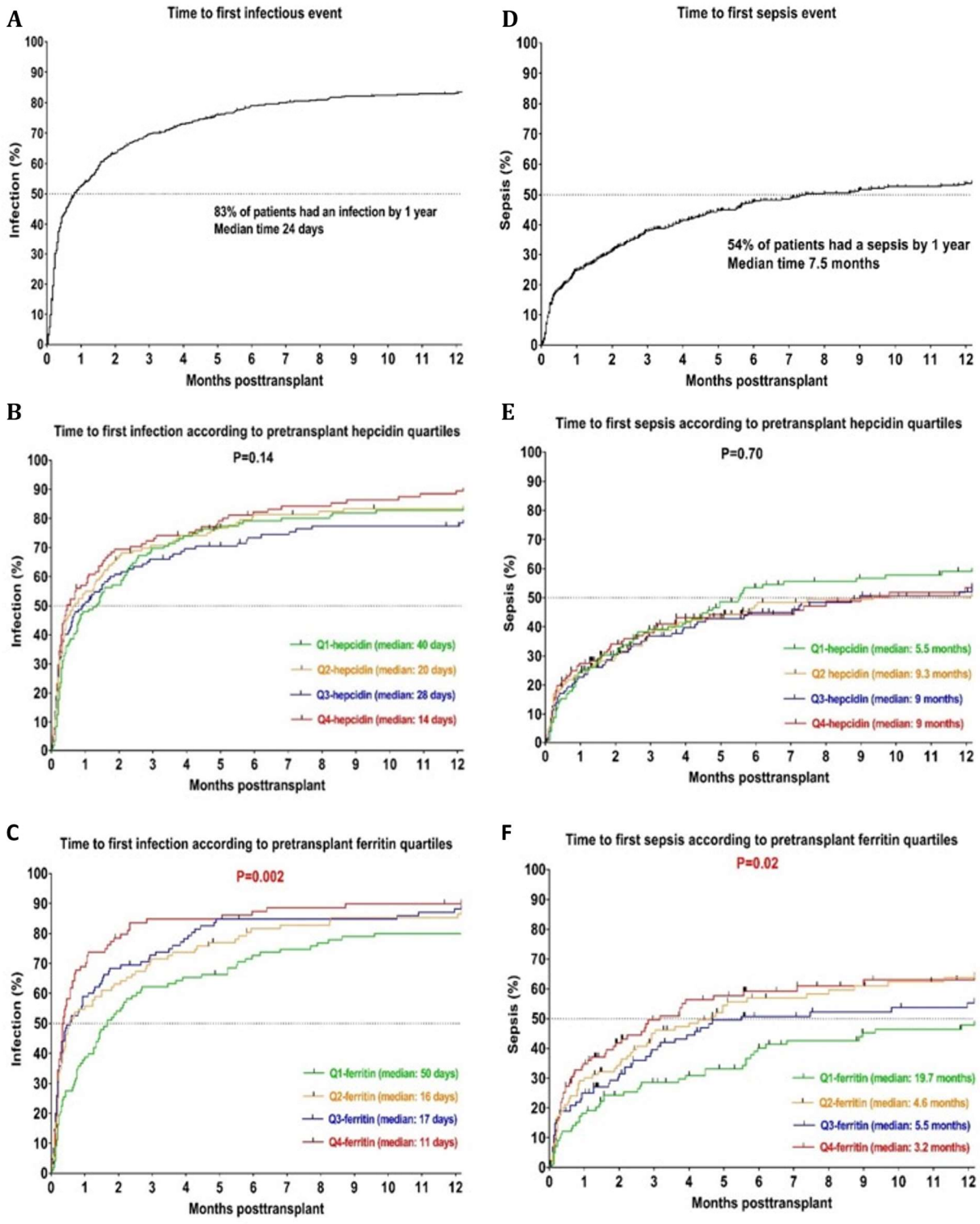


**Figure 6:** Time to first infection (A, B, C) or first sepsis (D, E, F) after allogeneic HSCT in all patients (A, D) as well as in patients stratified by pretransplant serum hepcidin quartiles (B, E,) or pretransplant serum ferritin quartiles (C, F).

**Table 11:** Cox proportional-hazard model of parameters (that had a p-value <0.15 in univariate analyses) impacting time to first infection (10.a), sepsis (10.b), CMV reactivation (10.c), zoster infection (10.d) and interstitial pneumonia (10.e). Other parameters were included into the model but were found not significant in multivariate analyses:

**For first infection (11.a):** age, PS, BMI, diagnosis, DRI, delay between diagnosis and transplant, number of treatment lines, transplant number, HCT-CI score, donor age, source of stem cells, graft manipulations, use of ATG, GVHD prophylaxis, mycotic prophylaxis, CD34+ cell dose; pretransplant RBC and platelet transfusions, ferritin (p=0.0003), serum iron, total bilirubin, CRP, uric acid, Hb, platelets, and lymphocytes.
**For first sepsis (11.b):** age, PS, diagnosis, delay between diagnosis and transplant, transplant number, HCT-CI score, source of stem cells, GVHD prophylaxis, antibiotic prophylaxis, CD34+ cell dose; pretransplant RBC and platelet transfusions, ferritin (p=0.05), and Tsat.
**For CMV reactivation/infection (11.c):** DRI, sex, donor age, source of stem cells, mycotic prophylaxis, CD34+ cell dose; pretransplant RBC transfusions, creatinine, Hb, and lymphocytes.
**For zoster infection (11.d**): age, HCT-CI score, conditioning, GVHD prophylaxis, antibiotic prophylaxis, CD34+ cell dose; pretransplant serum iron, Tsat, total bilirubin, CRP, and uric acid.
**For interstitial pneumonia (11.e):** age, DRI, HCT-CI score, source of stem cells, transplant number, CD34+ cell dose, graft manipulations, conditioning, use of ATG, mycotic prophylaxis; pretransplant ferritin (p=0.004), serum iron, Tsat, total bilirubin, ALAT, Hb and neutrophils.

| 11.a Multivariate analysis of time to first infection | | | | |
|---|---|---|---|---|
| **Significant parameters** | **P value** | **HR** | **95% CI** | |
| **Pretransplant Tsat** | 0.03 | 1.25 | 1.03 | 1.53 |
| **Type of conditioning (NMA vs MA)** | <0.0001 | 0.17 | 0.12 | 0.25 |
| **Sex (F vs M)** | 0.045 | 1.30 | 1.01 | 1.68 |
| **HLA-matched unrelated vs HLA identical sibling donor** | 0.03 | 1.41 | 1.03 | 1.93 |
| **HLA-mismatched unrelated vs HLA identical sibling donor** | <0.0001 | 1.91 | 1.38 | 2.63 |
| **Use of antibiotic prophylaxis** | 0.03 | 0.69 | 0.50 | 0.96 |

| 11.b Multivariate analysis of time to first sepsis | | | | |
|---|---|---|---|---|
| **Significant parameters** | **P value** | **HR** | **95% CI** | |
| **Pretransplant creatinine** | 0.008 | 1.69 | 1.15 | 2.50 |
| **Type of conditioning (NMA vs MA)** | <0.0001 | 0.50 | 0.36 | 0.70 |
| **HLA-mismatched unrelated vs HLA identical sibling donor** | <0.0001 | 2.28 | 1.52 | 3.41 |
| **Graft manipulations** | 0.002 | 1.76 | 1.23 | 2.52 |

| 11.c Multivariate analysis of time to CMV reactivation or infection | | | | |
|---|---|---|---|---|
| **Significant parameters** | **P value** | **HR** | **95% CI** | |
| **Age** | 0.006 | 1.02 | 1.00 | 1.03 |
| **CMV: Don neg & Rec + vs Don & Rec neg** | <0.0001 | 29.57 | 10.81 | 80.91 |
| **CMV: Don + & Rec neg vs Don & Rec neg** | 0.0001 | 8.92 | 2.91 | 27.39 |
| **CMV : Don & Rec + vs Don & Rec neg** | <0.0001 | 36.40 | 13.31 | 99.53 |

| 11.d Multivariate analysis of time to zoster infection | | | | |
|---|---|---|---|---|
| **Significant parameters** | **P value** | **HR** | **95% CI** | |
| **Bone marrow manipulations** | 0.03 | 1.95 | 1.07 | 3.57 |
| **Cord blood (± bone marrow/PBSC) vs PBSC (± bone marrow)** | 0.0002 | 5.79 | 2.32 | 14.45 |

| 11.e Multivariate analysis of time to interstitial pneumonia | | | | |
|---|---|---|---|---|
| **Significant parameters** | **P value** | **HR** | **95% CI** | |
| **GVHD prophylaxis (CNI ± MMF vs CNI ± MTX)** | 0.0002 | 0.24 | 0.11 | 0.50 |
| **CMV: Don neg & Rec + vs Don & Rec neg** | 0.004 | 4.26 | 1.60 | 11.32 |
| **CMV: Don & Rec + vs Don & Rec neg** | 0.006 | 4.02 | 1.48 | 10.87 |

*ATG*: anti-thymocyte globulin; *BMI*: body mass index; *CMV*: cytomegalovirus; *CNI*: calcineurin inhibitor; *CRP*: C reactive protein; *DRI*: disease risk index; *Don*: donor; *F*: female; *GVHD*: graft-versus-host disease; *HCT-CI*: comorbidity score; *HLA*: human leukocyte antigen; *MA*: myeloablative; *M*: male; *MMF*: mycophenolate mofetil; *MTX*: methotrexate; *NMA*: nonmyeloablative; *PBSC*: peripheral blood stem cells; *PS*: performance status; *RBC*: red blood cells; *Rec*: recipient; *Tsat*: transferrin saturation.

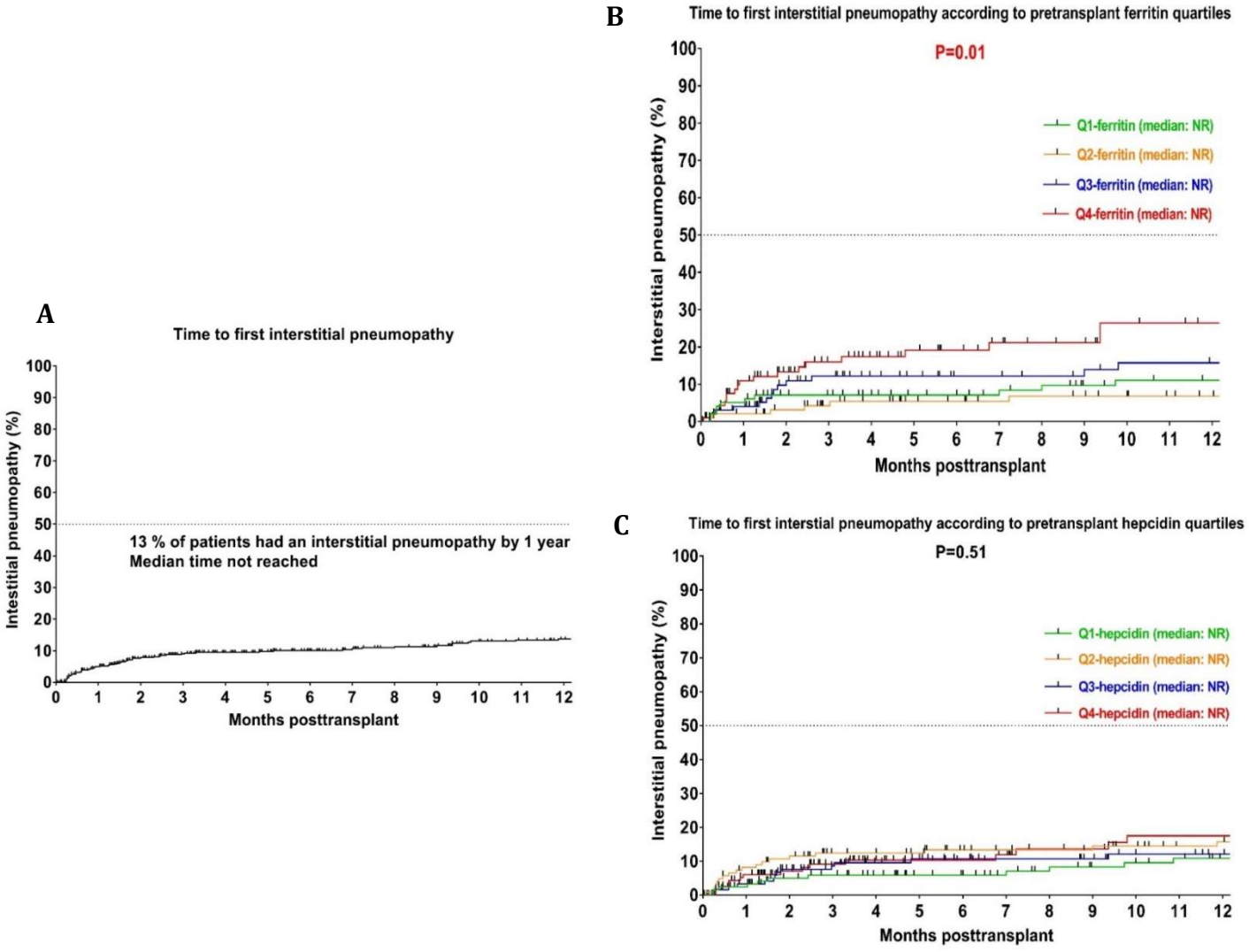


**Figure 7:** Time to first interstitial pneumopathy after allogeneic HSCT in all patients (A) as well as in patients stratified by pretransplant serum hepcidin quartiles (B) or pretransplant serum ferritin quartiles (C).

*Secondary malignancies*

The cumulative incidence of secondary malignancies was 8 %, with a median onset of 13.5 years among 42 affected patients. No association between pre-conditioning hepcidin or SF was found with development of secondary malignancies in univariate and multivariate analyses.

*GVHD*

The cumulative incidence of grade II-IV acute GVHD was 37% (median not reached), and that of chronic GVHD was 53% (median 11.2 months) at 1 year (**Figure 8A and 8D**). No difference in the incidence of acute GVHD was demonstrated between hepcidin or SF quartiles (**Figure 8B and 8C**). Similarly, no difference in chronic GVHD was found between the Q-hepcidin subgroups (**Figure 8E**). Surprisingly, there was a trend (p=0.053) for a higher incidence of chronic GVHD in the Q1-SF group (**Figure 8F**).

In univariate Cox proportional-hazard models, no significant correlation was shown between pre-conditioning hepcidin and the rates of acute or chronic GVHD nor between pre-conditioning SF and the incidence of acute GVHD. Pre-conditioning SF predicted for a reduced risk of chronic GVHD in univariate (HR: 0.87, p=0.02) but not multivariate analyses. Factors significantly associated with acute and chronic GVHD in multivariate analyses are displayed in **Tables 12.a and 12.b**, respectively.

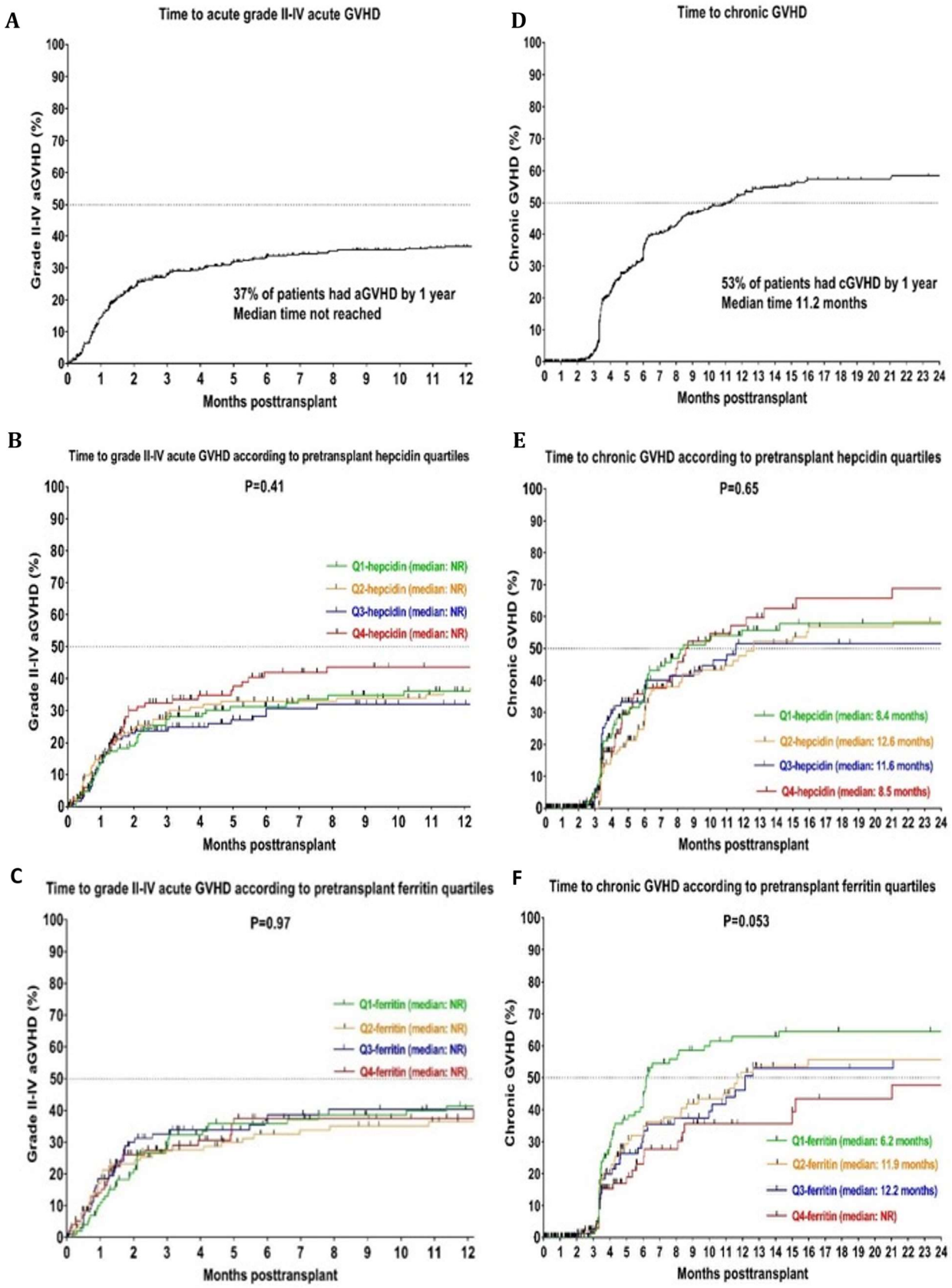


**Figure 8:** Time to develop grade II-IV acute graft-versus-host disease (GVHD) (A, B, C) or chronic GVHD (D, E, F) after allogeneic-HSCT in all patients (A) as well as in patients stratified by pretransplant serum hepcidin quartiles (B) or pretransplant serum ferritin quartiles (C).

**Table 12**: Cox proportional-hazard model of parameters (that had a p-value <0.15 in univariate analyses) impacting time to grade II-IV acute GVHD (11.a) or chronic GVHD (11.b). Other parameters were included into the model but were found not significant in multivariate analyses:

**For acute GVHD (12.a):** PS, DRI score, sex, number of treatment lines, donor age, graft manipulations, GVHD prophylaxis, antibiotic prophylaxis; pretransplant serum iron, and Hb.
**For chronic GVHD (12.b):** BMI, diagnosis, delay between diagnosis and transplant, number of treatment lines, HCT-CI score, source of stem cells, donor type and donor age, conditioning, GVHD prophylaxis, mycotic prophylaxis; pretransplant ferritin (p=0.02), serum iron, Tsat, total bilirubin, ALAT, creatinine, and uric acid.

| 12.a Multivariate analysis of grade II-IV acute GVHD | | | | |
|---|---|---|---|---|
| **Significant parameters** | **P value** | **HR** | **95% CI** | |
| **Pretransplant lymphocytes** | 0.002 | 0.49 | 0.31 | 0.76 |
| **Use of ATG** | 0.02 | 0.51 | 0.30 | 0.88 |
| **HLA-mismatched related vs HLA-identical sibling donor** | 0.01 | 3.06 | 1,27 | 7.38 |
| **HLA-matched unrelated vs HLA-identical sibling donor** | <0.0001 | 4.65 | 2.70 | 8.00 |
| **HLA-mismatched unrelated vs HLA-identical sibling donor** | <0.0001 | 4.77 | 2.68 | 8.47 |
| **Mycotic prophylaxis (other than fluconazole vs fluconazole)** | 0.0001 | 2.04 | 1.42 | 2.92 |

| 12.b Multivariate analysis of chronic GVHD | | | | |
|---|---|---|---|---|
| **Significant parameters** | **P value** | **HR** | **95% CI** | |
| **Graft manipulations** | 0.0008 | 0.49 | 0.32 | 0.74 |
| **Age** | 0.01 | 1.01 | 1.00 | 1.03 |
| **Transplant number** | 0.02 | 1.52 | 1.08 | 2.13 |

*ALAT:* alanine amino transferase*; ATG*: anti-thymocyte globulin; *BMI*: body mass index; *CI*: confidence interval; *DRI*: disease risk index; *GVHD*: graft-versus-host disease; *HCT-CI*: comorbidity score; *HLA*: human leukocyte antigen; *HR*: hazard ratio; *PS*: performance status; *Tsat*: transferrin saturation.

## 2.4 Discussion

The impact of pre-conditioning SF and hepcidin on outcomes following allo-HSCT has yielded conflicting results, sparking a debate (8, 9, 17, 20, 40, 69, 70). SF has often served as a surrogate marker for IO, but it can be influenced by various confounding factors, such as inflammation and liver disease (71). Indeed, a meta-analysis comprising four prospective studies that quantified IO through MRI in 276 patients prior to allo-HSCT showed no significant impact on OS or NRM, with the exception of higher NRM among IO patients undergoing allo-HCT with a RIC regimen (47). Since hepcidin plays a central role in the regulation of iron homeostasis, several studies have also analyzed the association between pre-conditioning serum hepcidin levels and various post-allo-HSCT complications. For instance, Sakamoto *et al.* observed a significant association between high pre-conditioning hepcidin levels and poor OS (61), but this was not confirmed by other investigators (58-60). The determinants of pre-conditioning SF and hepcidin, as well as their comprehensive impact on outcomes, remain relatively unexplored. In the initial part of our study, we investigated hepcidin regulation within the context of allo-HSCT, considering a variety of parameters of iron metabolism, and confirmed a strong positive correlation between pre-conditioning hepcidin and pre-conditioning SF (13). Furthermore, in contrast to other pathological situations, we demonstrated that during allo-HSCT, the activating effect of iron status dominated the inhibitory effect of erythropoiesis on hepcidin production (13).

***Determinants of pre-conditioning hepcidin and SF***

Four previous studies, involving patient cohorts ranging from 16 to 166 individuals, have explored the significance of serum hepcidin levels prior to transplantation (58-61). However, some of them (59, 60) measured serum hepcidin by commercial ELISA, which are known to be less reliable compared to mass spectrometry. In three studies, serum hepcidin was higher in pretransplant patients compared with normal controls (60, 61, 72), but this was not the case in the report by Kanda *et al.* (58). Our study is the largest to date, encompassing 502 allo-HSCT patients, and we also found elevated serum hepcidin values before transplantation. Sakamoto *et al.* (n=166, r=0.57, p<0.0001) (61) and Armand *et al.* (n=39, r=0.70) (72) demonstrated a positive correlation between pre-conditioning hepcidin and SF, and Kanda *et al.* reported significantly higher SF values in patients with high hepcidin (58), but this was not a reproducible finding in the two studies using ELISA (59, 60). We confirm here a robust positive correlation between pre-conditioning SF and hepcidin levels in more than 400 patients (Figure 1). Armand *et al.* also observed a positive correlation between, on the one hand, pre-conditioning hepcidin and, on the other hand, various factors including previous RBC transfusions (r=0.65), LIC (measured by MRI) (r=0.53), and CRP (r=0.39) (72). Eisfeld *et al.* reported that high hepcidin levels in AML patients before transplantation correlated with the number of previous RBC transfusions and low Hb

values, but not with age, sex, liver or renal function, or the number of previous lines of chemotherapy (60). Kanda *et al.* reported no significant relationship between hepcidin and age, sex, disease, disease risk, CRP, or reticulocytes (58), while Aki *et al.* found no association with CRP or interleukin-6 (59). However, none of these reports included multivariate analyses, leaving the significance of elevated hepcidin values somewhat elusive. In our study, univariate analysis revealed that patients with the highest hepcidin values had a significantly poorer PS, a higher HCT-CI score, more frequently a diagnosis of AL, a larger number of previous treatment lines, and more prior RBC and platelet transfusions. They also featured lower Hb, neutrophils, lymphocytes, platelets, and albumin, along with higher SF, serum iron, Tsat, CRP, and ALAT levels. Our extensive multivariate analysis on this substantial cohort revealed that pre-conditioning hepcidin is influenced not only by the number of RBC transfusions, but also by the HCT-CI and PS scores, as well as inflammation (neutrophil count and CRP). When SF is added to the list of covariates, it also stands out as a significant predictor of hepcidin values.

Many previous studies have reported elevated SF values before transplantation. Several of them found significant associations of SF with pretransplant clinical or biological patient characteristics in univariate correlation or group analyses. For instance, higher SF levels have been found in patients with elevated LIC values (29, 33, 60, 70, 73-75), serum iron or Tsat (37, 73, 74), CRP or interleukin-6 (24, 26, 42, 59, 76), ALAT (24), or low Hb (60), but these findings are not reproducible in other studies for CRP (21, 73) or Tsat (70). Male sex (73, 75), age (26), AL (26, 31, 34, 36, 73), number of previous treatment lines (31, 34), more advanced disease status (34, 36, 77), transfusion dependence or number of previous RBC transfusions (29, 33, 60, 70, 73-75), previous IFI (76), high HCT-CI (23, 26, 29, 74, 76), poor PS (23, 26, 29, 74, 76), but not the EBMT score (76), have been reported in association with high pre-conditioning SF. Nevertheless, there is no report that examined the significance of pre-conditioning SF in multivariate analysis. This may in part be explained by the difficulty to retrospectively collect the number of previous RBC transfusions, a factor strongly determining the importance of iron overload before transplantation. We managed to collect this information in our large cohort of patients to perform reliable multivariate analyses considering this indispensable parameter. We indeed confirmed markedly elevated SF levels at time of transplantation (1,622 ± 2,344 ng/mL). Patients with the highest SF values had the same characteristics as those with the highest hepcidin values, except for the HCT-CI score; they additionally had more advanced disease and a shorter time between diagnostic and transplantation. In our multiple linear regression analysis, pre-conditioning SF correlated positively with the degree of anemia, Tsat, liver (ALAT) and renal (creatinine) dysfunction, number of previous RBC and platelet transfusions, a diagnosis of AL (versus lymphoid malignancies or MPN) and the PS score. Pre-conditioning hepcidin was also associated with SF in a secondary analysis adjusting for this covariate. Notably, CRP as well as other factors,

such as age, sex, BMI, number of previous treatment lines, transplant number, DRI, HTC-CI, serum iron, platelets, neutrophils, lymphocytes, total bilirubin, and uric acid, were also found to be associated with elevated SF in univariate but no longer in multivariate analyses.

We observed an almost 4-fold surge of SF values in the first 100 days after transplantation. The average value of this peak was 5,730 ± 10,935 ng/ml, with a median of 3,419 ng/ml (range 76-168,306), which was reached after a median of 42 days. This peak strongly correlated with pre-conditioning hepcidin or SF but was observed in all their quartiles (**Table 3**). In our multivariate analysis, the SF peak was explained by a combination of factors, i.e. iron overload, marrow aplasia, inflammation, hepatic and renal dysfunction, transplant number and use of HLA- and ABO-mismatched donors (**Table 4.c**). Other factors were also significantly associated with the ferritin peak in univariate analyses but did not confirm in multivariate regression analyses (**Table 4**). Therefore, the determinants of the ferritin peak mostly confirm those of pre-conditioning SF. When included in secondary analyses, the ferritin peak also predicted for poor OS (HR 1.42, p<0.0001), poor PFS (HR 1.25, p=0.0002) and high NRM (HR 1.67, p<0.0001) at all timepoints in univariate but not in multivariate analyses (data not shown).

These findings highlight the complex interplay of multiple factors that impact SF levels both before and after transplantation. Pre-conditioning SF levels are influenced not only by the number of prior RBC transfusions resulting in iron overload in the liver and other organs, but also by a combination of biological and clinical factors that reflect patient comorbidities or the toxicity of previous treatments (liver damage, renal failure, and inflammation) and the severity of the disease. Pretransplant comorbidities and disease severity, in turn, can drive a greater need for treatments and transfusions.

***Impact of SF and hepcidin on survival***

Our next objective was to evaluate the influence of pre-conditioning SF and hepcidin levels on outcomes following allo-HSCT. We undertook this assessment through investigation of differences between subgroups based on hepcidin and SF quartiles as well as univariate and multivariate Cox proportional-hazard models.

Our findings unveiled that high levels of hepcidin (Q4, >46.36 ng/ml) and SF (Q4, >1,933 ng/ml) were associated with significantly poorer OS and PFS (**Figure 2**) or increased NRM. Univariate analyses indicated that both parameters were significantly associated with reduced OS and PFS and increased 100-day NRM, while only pre-conditioning SF was significantly associated with longer-term NRM. However, SF consistently demonstrated greater predictive power than hepcidin in bivariate analyses, and hepcidin did not retain its predictive value in multivariate analyses of OS, PFS or NRM. Three (58-61, 72) out of four previous studies failed to identify such an impact of pre-conditioning hepcidin on OS and all studies that analyzed it also failed to detect

an impact on PFS (58-61, 72) or NRM (58-61, 72). Only Sakamoto *et al.* (61) observed a significant association between high pre-conditioning hepcidin levels (>35 ng/ml) and poor OS, which persisted in multivariate analyses together with male sex and disease status, but not high SF. Although our study is by far the largest, hepcidin did not confirm its predictive value in multivariate analyses for day 100, 1-year or long-term survival (**Table 5**).

Our findings of a high predictive value of pre-conditioning SF align with previous reports, supporting the negative impact of pre-conditioning SF on OS, PFS and NRM. Indeed, many previous publications report this prediction of poor OS by elevated SF in UV (17, 22, 28, 29, 61, 76), or both in UV and MV (20, 21, 23, 24, 26, 27, 30, 31, 34-42, 69, 70, 73, 75, 77-84) analyses, while other studies did not (9, 33, 59, 60, 74, 85-90) or only in UV but not in MV analyses (17, 29, 61, 76) or only in subgroups in MV analyses (20, 21, 23, 24, 26, 27, 30, 31, 34-42, 69, 70, 73, 75, 77-83).

Elevated SF levels predicted much less for poor PFS or high relapse risk in both UV and MV analyses (24, 27, 28, 31, 36-39, 80, 82-84), as most studies did not find any association (9, 20, 26, 29, 32, 33, 35, 40, 60, 61, 74-76, 78, 87, 88, 90) or only in UV and not in MV analyses (17, 34) or only in subgroups in MV analyses (20, 21, 23, 24, 26, 27, 30, 31, 34-42, 69, 70, 73, 75, 77-83). It is noteworthy that studies demonstrating a significant association between pretransplant SF and relapse primarily focused on patients with AL (77, 80, 82-84, 87, 90). In our entire cohort, SF did not impact relapse in univariate analysis, and relapse rates were not different between SF quartiles. However, among patients with AL, we observed a higher rate of relapse in those with pretransplant SF, but not hepcidin, above compared to those below the median. Pretransplant SF was significantly associated with relapse in univariate cox models, but the DRI score was the only predictive factor for relapse in multivariate analyses. We did not confirm previous report showing a correlation between high SF and adverse cytogenetics in AL (91, 92).

Pretransplant hepcidin and SF levels were also higher in AL compared to other patients. Elevated serum hepcidin levels have been described at diagnosis and before/after HSCT in two small cohorts of AML patients (60, 93). SF levels have been reported to follow the leukemic burden in AML, being elevated at initial diagnosis, normalizing during remission, but increasing again at relapse (91, 94, 95). Elevated SF has been attributed to impaired erythropoiesis and inflammation, which are exacerbated by RBC transfusions after chemotherapy, creating an environment conducive to ferroptosis in AML cells (95). Ferroptosis refers to a form of cell demise triggered by deregulated lipid peroxidation and consequential membrane damage (96). The metabolic characteristics, genetic mutation patterns and mitochondrial dependency of AML cells greatly enhance their susceptibility to ferroptosis, but AML cells can employ various strategies to evade ferroptosis and maintain survival (97). On the other hand, some data suggest a correlation between HFE gene mutations, associated with genetic hemochromatosis due to insufficient

hepcidin production, and predisposition to develop AL (98-100). Although we did not explore the presence of HFE gene mutations in our cohort, it would be interesting to investigate whether the presence of such mutations could enhance iron stores and/or be associated with an increased risk of relapse or reduced survival.

For NRM, high SF values foretold high NRM in a minority of studies as some of them were positive in UV (31, 35, 77) or both in UV and MV (20, 21, 26, 27, 30, 36, 40, 42, 79) analyses , while the majority was negative both in UV and MV (9, 23, 28, 29, 32-34, 60, 61, 74-76, 78, 80, 82-84, 89, 90), or positive in UV but negative in MV (17, 24, 38, 41, 70) analyses. A meta-analysis of 25 studies, almost all of them retrospective and with a total of 4545 patients enrolled between 1988 and 2013, confirmed that high pre-conditioning SF is strongly associated with poor OS (HR 1.82; 95% CI 1.47–2.26; p<0.001) and PFS (HR 1.72; 95% CI 1.27–2.33; p<0.001) and increased NRM (HR 2.28; 95% CI 1.79–2.89; p<0.001) (8).

The array of parameters analyzed in our study unveiled predictors that were rarely considered in investigations by others. Indeed, the literature review shows that limiting the range of factors analyzed to a few known predictors in addition to SF itself may result in pre-conditioning SF being more often incorporated in the final predictive model while ignoring potentially better clinical or biological markers. We took a less restrictive approach for the selection of potential predictors of outcomes. For instance, pre-conditioning total bilirubin and type of mycotic prophylaxis emerged as reproducible predictors of outcomes in most of our models (**Tables 5, 6 and 7**). Pretransplant liver function indeed influenced pre-conditioning SF values and the inclusion of total bilirubin in the stepwise analysis may have resulted in pre-conditioning SF not being incorporated, although SF retained additional value for the prediction of long-term OS (**Table 5**) or short- and mid-term PFS (**Table 6**). Mycotic prophylaxis was based on itraconazole in earlier transplants, later replaced by fluconazole. Hence, the association of fluconazole use with short- and mid-term OS, PFS and NRM, may simply represent general progress in transplant outcomes over time but this was not specifically studied. Alternatively, as itraconazole brings about considerably more drug interactions than fluconazole, increased drug toxicity (in particular with cyclosporine that was only used in the itraconazole era) may have contributed to increased OS and NRM, but this remains speculative. Analogously, the incorporation of pretransplant RBC transfusions probably excluded pre-conditioning SF from multivariate models of NRM, because they are strongly correlated with each other. These findings exactly match those reported by Alessandrino *et al.* (33) and Cremers *et al.* (90) who also identified pre-conditioning SF and pretransplant RBC transfusions as risk factors for NRM, but only pretransplant RBC transfusions stood out in multivariate analyses. Finally, CRP has also been identified as predictor of inferior OS in some reports (26, 42), but most studies did not examine its potential impact. In our investigations, pre-

conditioning CRP predicted outcomes only in univariate analyses, but day 0 CRP emerged as an adverse prognostic factor in secondary analyses of OS at each time point.

Discrepant results among studies may also in part relate to the duration of follow-up. Indeed, in our multivariate analysis, pre-conditioning SF remained an independent marker of long-term mortality (HR 1.17, p=0.01) but this was not the case for 100-day or 1-year OS (**Table 5**). Conversely, our multivariate analysis showed that pre-conditioning SF was an independent predictor of poor PFS at 100 days (HR 1.25, p=0.04) or 1 year (HR 1.20, p=0.02) but not in the long term (**Table 6**). In secondary analyses, SF values on day 0 were likewise predictive of short- and long-term OS, as well as of short- and mid-term PFS. Fingrut *et al.* also observed that posttransplant rather than pre-conditioning SF had a sizable impact on OS and NRM (9), but other studies did not look at the prognostic significance of posttransplant SF levels. Such differences between predictive models of short-, mid- and long-term survival were not limited to pre-conditioning SF. The only parameters that retained significance across all three time periods were pre-conditioning total bilirubin for OS and PFS (not included in the long-term NRM model), type of mycotic prophylaxis for PFS and NRM (not included in the long-term OS model) and number of treatment lines for PFS. The PS score was also relevant for many outcomes and periods. Contrarywise, the DRI was important for late but not early OS and PFS outcomes, while the impact of donor HLA matching was restricted to early versus late timelines depending on the outcome being analyzed.

Other studies have also identified the DRI or similar parameters (diagnosis, disease status, number of treatment lines, advanced disease, prior transplant) alongside SF as powerful predictors of relapse, PFS, or OS after allogeneic transplantation (20, 24, 29, 31, 32, 34, 36, 42, 61, 69, 75, 77). Some of these variables (age, DRI, and SF) have been included in a prognostic scoring system proposed by Armand *et al.* for 445 patients with AL or MDS undergoing allo-HSCT (19). Indeed, the authors projected a scoring system based on five variables (age, disease type, disease stage at transplantation, cytogenetics, and pre-conditioning SF) that categorized patients into three groups with 5-year OS rates of 56% (low risk), 22% (intermediate risk), and 5% (high risk). Artz *et al.* constructed a score based on 3 simple biomarkers in a large cohort of 784 patients, i.e. pre-conditioning CRP, SF and albumin, leading to 3 groups with 1-year OS of 74%, 67% and 56%, respectively (26). In a cohort of 602 patients, Chee *et al.* aimed at enhancing the predictive capability of the DRI by incorporating 3 biomarkers, i.e. pre-conditioning SF ≥1000 µg/L (HR 1.94), Hb <10 g/dL (HR 1.71) and albumin <30 g/L (HR 2.65) (27). They established four prognostic groups for OS, relapse and NRM, and these models retained their validity at 100 days, 12 and 24 months post-transplantation.

As SF levels are the result of a combination of multiple determinant factors identified by our investigations, a remaining open question is whether iron overload per se, the presumably major

cause of elevated SF, is associated with poor outcomes after allogeneic transplantation. One approach to answering this question has consisted in prospectively measure the iron content of the liver (LIC) by MRI before conditioning. Although a meta-analysis by Armand *et al.* concluded that LIC had no significant impact on OS or NRM, except for higher NRM in IO patients undergoing allo-HCT with a RIC regimen (78, 79), the detailed results of the four studies are not uniform. Indeed, two prospective studies quantifying IO by MRI in 45 and 88 patients prior to allo-HSCT showed no significant impact on OS, relapse, NRM, VOD, organ failure, infections, aGVHD nor cGVHD (78, 79), while two others showed that high LIC was associated with higher NRM and poorer OS (74) or with increased infections but decreased incidences of aGVHD or cGVHD (101). In our analysis of survival outcomes in four subgroups based on pretransplant high/low SF and Tsat, the two subgroups with high SF values ("Low Tsat-High SF" and "High Tsat-High SF") exhibited the worst prognosis in terms of OS, PFS, and NRM, but not for relapse. These findings support the notion that SF remains a prognostic indicator, regardless of Tsat levels, which may reflect inflammation or other factors rather than actual IO. The true contribution of tissue iron to posttransplant survival and complications remains to be further studied.

Pretransplant total bilirubin was surprisingly associated with most survival outcomes in our multivariate analyses. Unfortunately, we did not collect data on unconjugated bilirubin and thus cannot comment on the relative contributions of the liver and hemolysis or ineffective erythropoiesis. However, in our multivariate model, pretransplant total bilirubin increased together with serum iron, ALAT, and a diagnosis of myeloproliferative neoplasm or non-malignant hematological disorders. To fully understand the significance of pretransplant bilirubin, it would be necessary to measure unconjugated bilirubin as well as markers of ineffective erythropoiesis such as GDF-15 (102, 103).

***Impact of SF and hepcidin on transfusions dependance and engraftment***

Large numbers of pretransplant RBC and platelet transfusions were associated with high pre-conditioning serum hepcidin and SF in univariate but not multivariate analyses. Pretransplant RBC and platelet transfusions were both mainly associated with the patient's age, PS, HCT-CI and disease type. Nevertheless, in bivariate analyses, pre-conditioning SF outperformed hepcidin as biomarker of previous RBC transfusions whereas both indicated platelet transfusion rates.

The impact of pre-conditioning SF or hepcidin on posttransplant transfusion requirements has been the subject of very limited investigations (59). In that study, elevated pre-conditioning SF, but not hepcidin, levels were associated with increased posttransplant RBC and platelet transfusion requirements, although no multivariate analysis was performed. Our study expands upon these limited investigations by analyzing the number of platelet and RBC transfusions received in the first 100 and 180 days post-transplantation, respectively. High pre-conditioning

SF, low pre-conditioning Hb, a diagnosis of MPN, poor PS, MA conditioning and the use of ATG were significantly associated with increased RBC transfusion needs (**Table 8.a**). In contrast, previous platelet transfusions, low pre-conditioning platelet counts, a diagnosis of MPN, MA conditioning, minor ABO incompatibility, and the use of MTX for GVHD prophylaxis, but not pre-conditioning SF, were linked to elevated platelet transfusion requirements (**Table 8.b**). The increased number of post-transplant RBC and platelet transfusions in MPN patients may be linked to splenomegaly and/or fibrosis delaying marrow recovery. The use of ATG has also been associated with higher transfusion needs in randomized studies (104, 105). Finally, we previously demonstrated the overall preservation of erythropoietic activity after NMA, resulting in fewer transfusions compared to MA conditioning (106).

No previous work examined the impact of SF on the time required to achieve transfusion independence after transplantation. In our study, high pre-conditioning SF predicted for delayed transfusion independence for both RBC and platelets and serum hepcidin only for platelets in univariate (**Figure 4**), but not multivariate (**Table 9**), analyses. Rather, poor PS, low CD34+ cell doses, major ABO incompatibility and inflammation slowed RBC transfusion independence (**Table 9.a**), while poor PS, low CD34+ cell doses, and the use of MTX as GVHD prophylaxis delayed platelet transfusion independence (**Table 9.b**).

Finally, our study showed that higher pre-conditioning SF levels were associated with longer times to neutrophil, platelet, and Hb recoveries in UV analyses (**Figure 5**). On the other hand, pre-conditioning hepcidin did not significantly impact the speed of hematological recovery, except for a borderline effect on platelet and Hb recoveries. None of these observations was confirmed in multivariate analyses. This is in agreement with two studies who found no association of pre-conditioning hepcidin with platelet or neutrophil engraftment (58, 59) whereas a third one found an association with delayed platelet recovery in MV analysis (61). While most studies have shown no association between pre-conditioning SF and neutrophil, platelet or RBC engraftment (28, 37, 40, 58) two found that SF was associated with slower neutrophil recovery in UV (40) or both UV and MV (75) analyses. High CRP levels were also associated with delayed platelet recovery. This may in part represent the impact of sepsis as an inducer of platelet consumption (107), as well as of viral infections such as CMV exercising cytotoxic effects on megakaryopoiesis (108). Moreover, antimicrobial treatments, such as high-dose ganciclovir for CMV infection, also cause thrombocytopenia (109). Infection may act through the induction of IFNs, which are often considered as negative regulators of cell proliferation and maturation (110). IFN-α inhibits the proliferation of hematopoietic progenitors and suppresses CFU-E, megakaryopoiesis and granulopoiesis (110). Although contradictory results were published on the impact of IFN-γ on the development of the megakaryocyte lineage, it may actually have dual potential by inhibiting or promoting the survival and growth of hematopoietic progenitors depending on the presence of

certain interactions, notably with stromal cell-derived factor 1 (111). The use of MTX and ABO incompatibility delayed engraftment across all cellular lineages, while the CD34+ cell dose and PS impacted only platelet and Hb engraftment, the use of ATG delayed only platelet and neutrophil recovery, and the use of cord blood, graft manipulations, CMV status and inflammation were relevant for only one of the three lineages (**Table 10**).

***Impact of SF and hepcidin on conditioning-related toxicities, infections and GVHD***

Our study found significant associations between certain conditioning-related toxicities and pre-conditioning SF (mucosal, cardiac, gastrointestinal, renal, and VOD) and hepcidin (renal and cutaneous toxicities only) in the univariate analysis (**Figure 3**). Pre-conditioning SF showed stronger predictive power for renal toxicity in the bivariate model, while in the multivariate analysis, pre-conditioning SF remained significant only for gastrointestinal toxicity. Hepcidin has been associated with mucositis but not with other toxicities (59). An increased incidence of mucositis has been found in patients with high SF before auto- but not allo-HSCT (18, 23) and an increased risk of cardiac toxicity has been described in some (112) but not in other (59, 74) studies. Previous studies have also reported an increased risk of liver toxicity and VOD with high pre-conditioning SF levels (22, 23, 43, 59, 71), but seldom in MV analyses (22, 71), while as many have not (59). It is of note that LIC was not predictive of VOD either (78, 79).

Previous studies have consistently shown that iron acquisition plays a crucial role in the growth and virulence of certain microorganisms, including Aspergillus species, leading to increased susceptibility to infections (113). While some prior research found a potential association between higher pre-conditioning serum hepcidin and rates of neutropenic fever (only in UV analysis) (59) or bacterial infection (58), but not CMV reactivation (58), while others did not (60). Our results demonstrate that hepcidin was not correlated with infections. Although bacterial proliferation is favored by increased iron levels, hepcidin itself acts as a protector against infections, these opposite changes potentially explaining the absence of an association. On the other hand, Tsat instead of SF was included in the multivariate model of infections (**Table 11**), potentially reflecting iron directly available to bacteria in the blood, rather than storage iron, which may influence the risk of infection. Some previous studies have found pre-conditioning SF to be predictive of bacterial (21, 40, 85), fungal (23, 25, 43, 114), or overall (20, 37, 40, 58, 59, 69, 70, 85) infections, as well as of idiopathic or clinical IP (115), but many others did not (18, 28, 30, 34, 74, 75). A large meta-analysis (8) suggested a possible link between elevated pre-conditioning SF levels and a trend for more bloodstream infections (OR 1.67, 95% CI 0.93–3.01; p=0.09). However, our study's findings align with existing data, indicating a significant association between high pre-conditioning SF levels and a higher risk of infectious events or IP (**Figure 6**) at 1 year, but not CMV reactivation nor zoster infection, although this did not hold true in MV analyses

(**Table 11**). Other factors were identified in our multivariate Cox models, i.e. CMV status and MTX for IP, CMV status and age for CMV, source of stem cell or graft manipulation for zoster, absence of quinolone prophylaxis, unrelated donors, female sex and MA conditioning for bacterial infections, and renal dysfunction, MA conditioning, HLA mismatch and graft manipulation for sepsis.

Our study found no significant association between pre-conditioning hepcidin or SF levels and the risk of acute or chronic GVHD (**Table 12 and Figure 8**), although pre-conditioning SF was associated with a decreased risk of cGVHD in UV analysis. This is consistent with most negative previous research on hepcidin (60, 61) and SF (9, 17, 20, 23, 26, 28, 30, 32, 34, 37, 42, 61, 69, 74, 75, 79). Only two studies found that, in UV analyses, high SF predicted for a low incidence of both aGVHD and cGVHD (31) or of cGVHD in AML patients only (42), while three others indicated an increased risk of severe aGVHD in UV analysis (24) or of cGVHD in MV analysis (24). In the meta-analysis of Yan *et al.*, high pre-conditioning SF was associated with a lower incidence of cGVHD (OR 0.74, 95% CI 0.58–0.96, $p<0.05$) but bore no significant relationship with aGVHD (OR 1.08, 95% CI 0.72–1.62). In our MV model, high pre-conditioning lymphocytes in the recipient and use of ATG protected against aGVHD, while unrelated or HLA-mismatched donors and mycotic prophylaxis other than fluconazole were associated with a higher risk of a GVHD. We can only offer speculative explanations for the association of antifungal prophylaxis other than fluconazole with aGVHD : it may reflect a potential effect of transplantation year, earlier transplants being potentially associated with suboptimal donor selection and more frequent MA conditioning; in addition, more difficult management of calcineurin inhibitors with itraconazole, in particular in the more than 40% of patients then on cyclosporine rather than tacrolimus, may have led to an elevated risk of aGVHD. Graft manipulations protected against cGVHD, while age, transplant number and predicted for more cGVHD. Iron and ferritin may act as immune regulators (116), suppressing T-cell proliferation (117), disrupting B-cell maturation (118), and inhibiting myeloid cell proliferation (119). Additionally, H-ferritin may induce the production of interleukin-10 by regulatory T cells, leading to immune response suppression (120). Further research is warranted to elucidate the potential immunomodulatory mechanisms of iron and ferritin in the context of GVHD.

Here again, the question of iron overload per se versus SF, which is determined by a number of different factors, has been approached by measuring LIC by MRI before conditioning. Elevated LIC has not been found to predict VOD or other toxicities, infections, aGVHD or cGHVD in three studies (74, 78, 79), but a last one found it associated with increased infections but lower incidences of acute or chronic GVHD (101).

It is worth noting that other studies examining the impact of hepcidin and SF in the context of allogeneic transplantation were mostly published 5-15 years ago. This may limit the relevance of

our findings on risk prediction in newer forms of allogeneic transplantation, such as haploidentical donor transplantation with post-transplant cyclophosphamide, also taking into considerations other advances in supportive care. Therefore, the relevance of our findings, derived from a large cohort with simultaneous evaluation of SF and hepcidin, will require further assessment in a more recent, multicenter, cohort of patients.

## 2.5 Conclusion

The impact of pre-conditioning SF and hepcidin on outcomes after allo-HSCTC continues to be a subject of debate, as conflicting results have been reported in the literature. In our study, we confirmed a strong positive correlation between pre-conditioning hepcidin and SF levels, which were influenced by a diverse array of biological and clinical factors. Elevated SF levels were associated with adverse outcomes, including lower OS and PFS, higher NRM, an increased risk of infectious events or of some specific toxicities, slower engraftment, and higher transfusion requirements. Although pre-conditioning hepcidin was linked to worse OS, lower PFS, increased NRM, and delayed transfusion independence, SF exhibited greater significance than hepcidin in predicting these outcomes. Nevertheless, these observations were predominantly not confirmed in multivariate analyses, except for explaining long-term OS, 100-day and 1-year PFS, as well as the number of posttransplant RBC transfusions. Our study provides valuable insights into the impact of pre-conditioning SF and hepcidin on allo-HSCT outcomes and emphasizes the importance of considering multiple biological and clinical variables in such analyses. Furthermore, we observed that both SF and hepcidin levels can be influenced by multiple factors, suggesting that abnormal SF or hepcidin levels may represent a non-specific predictor of transplant outcomes. While simplified indexes may find SF values useful for deriving predicting scores, a more thorough assessment of major clinical and biological features probably provides better prediction of outcomes.

Despite these advancements, certain limitations, such as patient disparities and potential collinearity in multivariable analyses, warrant further investigation to enhance our understanding of these complex relationships. These findings underscore the intricacy of iron metabolism and its influence on outcomes after allo-HSCT, highlighting the need for further research to unravel the underlying mechanisms and explore potential therapeutic interventions.

## 2.6 References


1. Styczyński J, Tridello G, Koster L, Iacobelli S, van Biezen A, van der Werf S, et al. Death after hematopoietic stem cell transplantation: changes over calendar year time, infections and associated factors. Bone Marrow Transplant. 2020;55(1):126-36.
2. Chang C, Storer BE, Scott BL, Bryant EM, Shulman HM, Flowers ME, et al. Hematopoietic cell transplantation in patients with myelodysplastic syndrome or acute myeloid leukemia arising from myelodysplastic syndrome: similar outcomes in patients with de novo disease and disease following prior therapy or antecedent hematologic disorders. Blood. 2007;110(4):1379-87.
3. Servais S, Beguin Y, Delens L, Ehx G, Fransolet G, Hannon M, et al. Novel approaches for preventing acute graft-versus-host disease after allogeneic hematopoietic stem cell transplantation. Expert Opin Investig Drugs. 2016;25(8):957-72.
4. Cutler CS, Koreth J, Ritz J. Mechanistic approaches for the prevention and treatment of chronic GVHD. Blood. 2017;129(1):22-9.
5. Einsele H, Ehninger G, Hebart H, Wittkowski KM, Schuler U, Jahn G, et al. Polymerase chain reaction monitoring reduces the incidence of cytomegalovirus disease and the duration and side effects of antiviral therapy after bone marrow transplantation. Blood. 1995;86(7):2815-20.
6. Horan JT, Logan BR, Agovi-Johnson MA, Lazarus HM, Bacigalupo AA, Ballen KK, et al. Reducing the risk for transplantation-related mortality after allogeneic hematopoietic cell transplantation: how much progress has been made? J Clin Oncol. 2011;29(7):805-13.
7. Sorror ML, Maris MB, Storb R, Baron F, Sandmaier BM, Maloney DG, et al. Hematopoietic cell transplantation (HCT)-specific comorbidity index: a new tool for risk assessment before allogeneic HCT. Blood. 2005;106(8):2912-9.
8. Yan Z, Chen X, Wang H, Chen Y, Chen L, Wu P, et al. Effect of pre-transplantation serum ferritin on outcomes in patients undergoing allogeneic hematopoietic stem cell transplantation: A meta-analysis. Medicine (Baltimore). 2018;97(27):e10310.
9. Fingrut W, Law A, Lam W, Michelis FV, Viswabandya A, Lipton JH, et al. Post-transplant ferritin level predicts outcomes after allogeneic hematopoietic stem cell transplant, independent from pre-transplant ferritin level. Ann Hematol. 2021;100(3):789-98.
10. Brissot E, Savani BN, Mohty M. Management of high ferritin in long-term survivors after hematopoietic stem cell transplantation. Semin Hematol. 2012;49(1):35-42.
11. Or R, Matzner Y, Konijn AM. Serum ferritin in patients undergoing bone marrow transplantation. Cancer. 1987;60(5):1127-31.
12. Konijn AM, Kaplan R, Or R, Matzner Y. Glycosylated serum ferritin in patients with hematological malignancies before and after bone marrow transplantation. Leuk Lymphoma. 1992;7(1-2):151-6.
13. Pirotte M, Fillet M, Seidel L, Jaspers A, Baron F, Beguin Y. Erythroferrone and hepcidin as mediators between erythropoiesis and iron metabolism during allogeneic hematopoietic stem cell transplant. Am J Hematol. 2021;96(10):1275-86.
14. Porter JB. Practical management of iron overload. Br J Haematol. 2001;115(2):239-52.
15. Camaschella C, Nai A. Ineffective erythropoiesis and regulation of iron status in iron loading anaemias. Br J Haematol. 2016;172(4):512-23.
16. Isidori A, Borin L, Elli E, Latagliata R, Martino B, Palumbo G, et al. Iron toxicity - Its effect on the bone marrow. Blood Rev. 2018;32(6):473-9.
17. Armand P, Kim HT, Cutler CS, Ho VT, Koreth J, Alyea EP, et al. Prognostic impact of elevated pretransplantation serum ferritin in patients undergoing myeloablative stem cell transplantation. Blood. 2007;109(10):4586-8.
18. Altes A, Remacha AF, Sarda P, Baiget M, Sureda A, Martino R, et al. Early clinical impact of iron overload in stem cell transplantation. A prospective study. Ann Hematol. 2007;86(6):443-7.
19. Armand P, Kim HT, Cutler CS, Ho VT, Koreth J, Ritz J, et al. A prognostic score for patients with acute leukemia or myelodysplastic syndromes undergoing allogeneic stem cell transplantation. Biol Blood Marrow Transplant. 2008;14(1):28-35.

20. Kataoka K, Nannya Y, Hangaishi A, Imai Y, Chiba S, Takahashi T, et al. Influence of pretransplantation serum ferritin on nonrelapse mortality after myeloablative and nonmyeloablative allogeneic hematopoietic stem cell transplantation. Biol Blood Marrow Transplant. 2009;15(2):195-204.
21. Kanda J, Mizumoto C, Ichinohe T, Kawabata H, Saito T, Yamashita K, et al. Pretransplant serum ferritin and C-reactive protein as predictive factors for early bacterial infection after allogeneic hematopoietic cell transplantation. Bone Marrow Transplant. 2011;46(2):208-16.
22. Maradei SC, Maiolino A, de Azevedo AM, Colares M, Bouzas LF, Nucci M. Serum ferritin as risk factor for sinusoidal obstruction syndrome of the liver in patients undergoing hematopoietic stem cell transplantation. Blood. 2009;114(6):1270-5.
23. Sucak GT, Yegin ZA, Ozkurt ZN, Aki SZ, Yağci M. Iron overload: predictor of adverse outcome in hematopoietic stem cell transplantation. Transplant Proc. 2010;42(5):1841-8.
24. Wahlin A, Lorenz F, Fredriksson M, Remberger M, Wahlin BE, Hägglund H. Hyperferritinemia is associated with low incidence of graft versus host disease, high relapse rate, and impaired survival in patients with blood disorders receiving allogeneic hematopoietic stem cell grafts. Med Oncol. 2011;28(2):552-8.
25. Dadwal SS, Tegtmeier B, Liu X, Frankel P, Ito J, Forman SJ, et al. Impact of pretransplant serum ferritin level on risk of invasive mold infection after allogeneic hematopoietic stem cell transplantation. Eur J Haematol. 2015;94(3):235-42.
26. Artz AS, Logan B, Zhu X, Akpek G, Bufarull RM, Gupta V, et al. The prognostic value of serum C-reactive protein, ferritin, and albumin prior to allogeneic transplantation for acute myeloid leukemia and myelodysplastic syndromes. Haematologica. 2016;101(11):1426-33.
27. Chee L, Tacey M, Lim B, Lim A, Szer J, Ritchie D. Pre-transplant ferritin, albumin and haemoglobin are predictive of survival outcome independent of disease risk index following allogeneic stem cell transplantation. Bone Marrow Transplant. 2017;52(6):870-7.
28. Kim YR, Kim JS, Cheong JW, Song JW, Min YH. Transfusion-associated iron overload as an adverse risk factor for transplantation outcome in patients undergoing reduced-intensity stem cell transplantation for myeloid malignancies. Acta Haematol. 2008;120(3):182-9.
29. Platzbecker U, Bornhäuser M, Germing U, Stumpf J, Scott BL, Kröger N, et al. Red blood cell transfusion dependence and outcome after allogeneic peripheral blood stem cell transplantation in patients with de novo myelodysplastic syndrome (MDS). Biol Blood Marrow Transplant. 2008;14(11):1217-25.
30. Lee JW, Kang HJ, Kim EK, Kim H, Shin HY, Ahn HS. Effect of iron overload and iron-chelating therapy on allogeneic hematopoietic SCT in children. Bone Marrow Transplant. 2009;44(12):793-7.
31. Mahindra A, Bolwell B, Sobecks R, Rybicki L, Pohlman B, Dean R, et al. Elevated pretransplant ferritin is associated with a lower incidence of chronic graft-versus-host disease and inferior survival after myeloablative allogeneic haematopoietic stem cell transplantation. Br J Haematol. 2009;146(3):310-6.
32. Mahindra A, Sobecks R, Rybicki L, Pohlman B, Dean R, Andresen S, et al. Elevated pretransplant serum ferritin is associated with inferior survival following nonmyeloablative allogeneic transplantation. Bone Marrow Transplant. 2009;44(11):767-8.
33. Alessandrino EP, Della Porta MG, Bacigalupo A, Malcovati L, Angelucci E, Van Lint MT, et al. Prognostic impact of pre-transplantation transfusion history and secondary iron overload in patients with myelodysplastic syndrome undergoing allogeneic stem cell transplantation: a GITMO study. Haematologica. 2010;95(3):476-84.
34. Lim ZY, Fiaccadori V, Gandhi S, Hayden J, Kenyon M, Ireland R, et al. Impact of pre-transplant serum ferritin on outcomes of patients with myelodysplastic syndromes or secondary acute myeloid leukaemia receiving reduced intensity conditioning allogeneic haematopoietic stem cell transplantation. Leuk Res. 2010;34(6):723-7.
35. Oakes R, Sood N, Pearce R, Swirsky D, Cook G, Gilleece M. Prognostic impact of serum ferritin concentration on survival following reduced-intensity conditioned allogeneic haemopoietic SCT. Bone Marrow Transplant. 2010;45(12):1754-5.

36. Tachibana T, Tanaka M, Numata A, Takasaki H, Ito S, Ohshima R, et al. Pretransplant serum ferritin has a prognostic influence on allogeneic transplant regardless of disease risk. Leuk Lymphoma. 2012;53(3):456-61.
37. Sivgin S, Baldane S, Kaynar L, Kurnaz F, Pala C, Ozturk A, et al. Pretransplant serum ferritin level may be a predictive marker for outcomes in patients having undergone allogeneic hematopoietic stem cell transplantation. Neoplasma. 2012;59(2):183-90.
38. Oran B, Kongtim P, Popat U, de Lima M, Jabbour E, Lu X, et al. Cytogenetics, donor type, and use of hypomethylating agents in myelodysplastic syndrome with allogeneic stem cell transplantation. Biol Blood Marrow Transplant. 2014;20(10):1618-25.
39. Duda K, Wieczorkiewicz-Kabut A, Spałek A, Koclęga A, Kopińska AJ, Woźniczka K, et al. Room for Improvement: A 20-Year Single Center Experience with Allogeneic Stem Cell Transplantation for Myelodysplastic Syndromes. Indian J Hematol Blood Transfus. 2022;38(4):680-90.
40. Barba P, Valcárcel D, Pérez-Simón JA, Fernández-Avilés F, Piñana JL, Martino R, et al. Impact of hyperferritinemia on the outcome of reduced-intensity conditioning allogeneic hematopoietic cell transplantation for lymphoid malignancies. Biol Blood Marrow Transplant. 2013;19(4):597-601.
41. Meyer SC, O'Meara A, Buser AS, Tichelli A, Passweg JR, Stern M. Prognostic impact of posttransplantation iron overload after allogeneic stem cell transplantation. Biol Blood Marrow Transplant. 2013;19(3):440-4.
42. Sakamoto S, Kawabata H, Kanda J, Uchiyama T, Mizumoto C, Kondo T, et al. Differing impacts of pretransplant serum ferritin and C-reactive protein levels on the incidence of chronic graft-versus-host disease after allogeneic hematopoietic stem cell transplantation. Int J Hematol. 2013;97(1):109-16.
43. Ozyilmaz E, Aydogdu M, Sucak G, Aki SZ, Ozkurt ZN, Yegin ZA, et al. Risk factors for fungal pulmonary infections in hematopoietic stem cell transplantation recipients: the role of iron overload. Bone Marrow Transplant. 2010;45(10):1528-33.
44. Altes A, Remacha AF, Sarda P, Baiget M, Canals C, Sierra J. The relationship between transferrin saturation and erythropoiesis during stem cell transplantation. Haematologica. 2006;91(7):992-3.
45. Carmine TC, Evans P, Bruchelt G, Evans R, Handgretinger R, Niethammer D, et al. Presence of iron catalytic for free radical reactions in patients undergoing chemotherapy: implications for therapeutic management. Cancer Lett. 1995;94(2):219-26.
46. Atilla E, Toprak SK, Demirer T. Current Review of Iron Overload and Related Complications in Hematopoietic Stem Cell Transplantation. Turk J Haematol. 2017;34(1):1-9.
47. Armand P, Kim HT, Virtanen JM, Parkkola RK, Itälä-Remes MA, Majhail NS, et al. Iron overload in allogeneic hematopoietic cell transplantation outcome: a meta-analysis. Biol Blood Marrow Transplant. 2014;20(8):1248-51.
48. Camaschella C, Nai A, Silvestri L. Iron metabolism and iron disorders revisited in the hepcidin era. Haematologica. 2020;105(2):260-72.
49. Ganz T. Hepcidin and iron regulation, 10 years later. Blood. 2011;117(17):4425-33.
50. Gao J, Chen J, Kramer M, Tsukamoto H, Zhang AS, Enns CA. Interaction of the hereditary hemochromatosis protein HFE with transferrin receptor 2 is required for transferrin-induced hepcidin expression. Cell Metab. 2009;9(3):217-27.
51. Armitage AE, Eddowes LA, Gileadi U, Cole S, Spottiswoode N, Selvakumar TA, et al. Hepcidin regulation by innate immune and infectious stimuli. Blood. 2011;118(15):4129-39.
52. Mayeur C, Lohmeyer LK, Leyton P, Kao SM, Pappas AE, Kolodziej SA, et al. The type I BMP receptor Alk3 is required for the induction of hepatic hepcidin gene expression by interleukin-6. Blood. 2014;123(14):2261-8.
53. Pinto JP, Ribeiro S, Pontes H, Thowfeequ S, Tosh D, Carvalho F, et al. Erythropoietin mediates hepcidin expression in hepatocytes through EPOR signaling and regulation of C/EBPalpha. Blood. 2008;111(12):5727-33.
54. Kautz L, Jung G, Valore EV, Rivella S, Nemeth E, Ganz T. Identification of erythroferrone as an erythroid regulator of iron metabolism. Nat Genet. 2014;46(7):678-84.

55. Jaspers A, Baron F, Willems E, Seidel L, Wiegerinck ET, Swinkels DW, et al. Serum hepcidin following autologous hematopoietic cell transplantation: an illustration of the interplay of iron status, erythropoiesis and inflammation. Haematologica. 2014;99(3):e35-7.
56. Arezes J, Foy N, McHugh K, Sawant A, Quinkert D, Terraube V, et al. Erythroferrone inhibits the induction of hepcidin by BMP6. Blood. 2018;132(14):1473-7.
57. Pak M, Lopez MA, Gabayan V, Ganz T, Rivera S. Suppression of hepcidin during anemia requires erythropoietic activity. Blood. 2006;108(12):3730-5.
58. Kanda J, Mizumoto C, Kawabata H, Ichinohe T, Tsuchida H, Tomosugi N, et al. Clinical significance of serum hepcidin levels on early infectious complications in allogeneic hematopoietic stem cell transplantation. Biol Blood Marrow Transplant. 2009;15(8):956-62.
59. Akı SZ, Paşaoğlu H, Yeğin ZA, Suyanı E, Demirtaş CY, Ozkurt ZN, et al. Impact of prohepcidin levels and iron parameters on early post-transplantation toxicities. Hematology. 2011;16(5):284-90.
60. Eisfeld AK, Westerman M, Krahl R, Leiblein S, Liebert UG, Hehme M, et al. Highly Elevated Serum Hepcidin in Patients with Acute Myeloid Leukemia prior to and after Allogeneic Hematopoietic Cell Transplantation: Does This Protect from Excessive Parenchymal Iron Loading? Adv Hematol. 2011;2011:491058.
61. Sakamoto S, Kawabata H, Kanda J, Uchiyama T, Mizumoto C, Kitano T, et al. High pretransplant hepcidin levels are associated with poor overall survival and delayed platelet engraftment after allogeneic hematopoietic stem cell transplantation. Cancer Med. 2017;6(1):120-8.
62. Bacigalupo A, Ballen K, Rizzo D, Giralt S, Lazarus H, Ho V, et al. Defining the intensity of conditioning regimens: working definitions. Biol Blood Marrow Transplant. 2009;15(12):1628-33.
63. Diepeveen LE, Laarakkers CMM, Martos G, Pawlak ME, Uğuz FF, Verberne KESA, et al. Provisional standardization of hepcidin assays: creating a traceability chain with a primary reference material, candidate reference method and a commutable secondary reference material. Clin Chem Lab Med. 2019;57(6):864-72.
64. Bearman SI, Appelbaum FR, Back A, Petersen FB, Buckner CD, Sullivan KM, et al. Regimen-related toxicity and early posttransplant survival in patients undergoing marrow transplantation for lymphoma. J Clin Oncol. 1989;7(9):1288-94.
65. Mohty M, Malard F, Abecassis M, Aerts E, Alaskar AS, Aljurf M, et al. Revised diagnosis and severity criteria for sinusoidal obstruction syndrome/veno-occlusive disease in adult patients: a new classification from the European Society for Blood and Marrow Transplantation. Bone Marrow Transplant. 2016;51(7):906-12.
66. Harris AC, Young R, Devine S, Hogan WJ, Ayuk F, Bunworasate U, et al. International, Multicenter Standardization of Acute Graft-versus-Host Disease Clinical Data Collection: A Report from the Mount Sinai Acute GVHD International Consortium. Biol Blood Marrow Transplant. 2016;22(1):4-10.
67. Jagasia MH, Greinix HT, Arora M, Williams KM, Wolff D, Cowen EW, et al. National Institutes of Health Consensus Development Project on Criteria for Clinical Trials in Chronic Graft-versus-Host Disease: I. The 2014 Diagnosis and Staging Working Group report. Biol Blood Marrow Transplant. 2015;21(3):389-401.e1.
68. Armand P, Kim HT, Logan BR, Wang Z, Alyea EP, Kalaycio ME, et al. Validation and refinement of the Disease Risk Index for allogeneic stem cell transplantation. Blood. 2014;123(23):3664-71.
69. Pullarkat V, Blanchard S, Tegtmeier B, Dagis A, Patane K, Ito J, et al. Iron overload adversely affects outcome of allogeneic hematopoietic cell transplantation. Bone Marrow Transplant. 2008;42(12):799-805.
70. Altès A, Remacha AF, Sureda A, Martino R, Briones J, Canals C, et al. Iron overload might increase transplant-related mortality in haematopoietic stem cell transplantation. Bone Marrow Transplant. 2002;29(12):987-9.
71. Goto T, Ikuta K, Inamoto Y, Kamoshita S, Yokohata E, Koyama D, et al. Hyperferritinemia after adult allogeneic hematopoietic cell transplantation: quantification of iron burden by determining non-transferrin-bound iron. Int J Hematol. 2013;97(1):125-34.

72. Armand P, Kim HT, Rhodes J, Sainvil MM, Cutler C, Ho VT, et al. Iron overload in patients with acute leukemia or MDS undergoing myeloablative stem cell transplantation. Biol Blood Marrow Transplant. 2011;17(6):852-60.
73. Bazuaye GN, Buser A, Gerull S, Tichelli A, Stern M. Prognostic impact of iron parameters in patients undergoing allo-SCT. Bone Marrow Transplant. 2012;47(1):60-4.
74. Wermke M, Schmidt A, Middeke JM, Sockel K, von Bonin M, Schönefeldt C, et al. MRI-based liver iron content predicts for nonrelapse mortality in MDS and AML patients undergoing allogeneic stem cell transplantation. Clin Cancer Res. 2012;18(23):6460-8.
75. Tanaka M, Kanamori H, Matsumoto K, Tachibana T, Numata A, Ohashi K, et al. Clinical significance of pretransplant serum ferritin on the outcome of allogeneic hematopoietic SCT: a prospective cohort study by the Kanto Study Group for Cell Therapy. Bone Marrow Transplant. 2015;50(5):727-33.
76. Akı Ş, Suyanı E, Bildacı Y, Çakar MK, Baysal NA, Sucak GT. Prognostic role of pre-transplantation serum C-reactive protein levels in patients with acute leukemia undergoing myeloablative allogeneic stem cell transplantation. Clin Transplant. 2012;26(5):E513-21.
77. Tachibana T, Tanaka M, Takasaki H, Numata A, Maruta A, Ishigatsubo Y, et al. Pre-SCT serum ferritin is a prognostic factor in adult AML, but not ALL. Bone Marrow Transplant. 2011;46(9):1268-9.
78. Armand P, Sainvil MM, Kim HT, Rhodes J, Cutler C, Ho VT, et al. Does iron overload really matter in stem cell transplantation? Am J Hematol. 2012;87(6):569-72.
79. Trottier BJ, Burns LJ, DeFor TE, Cooley S, Majhail NS. Association of iron overload with allogeneic hematopoietic cell transplantation outcomes: a prospective cohort study using R2-MRI-measured liver iron content. Blood. 2013;122(9):1678-84.
80. Boehm A, Sperr WR, Kalhs P, Greinix H, Valent P, Worel N, et al. Long-term follow-up after allogeneic stem cell transplantation in patients with myelodysplastic syndromes or secondary acute myeloid leukemia: a single center experience. Wien Klin Wochenschr. 2014;126(1-2):23-9.
81. Matsumoto K, Yamamoto W, Ogusa E, Sugimoto E, Maruta A, Ishigatsubo Y, et al. Impact of pretransplant serum ferritin on outcome in adult patients receiving cord blood transplant for acute leukemia. Leuk Lymphoma. 2014;55(2):460-1.
82. Tanaka M, Tachibana T, Numata A, Takasaki H, Matsumoto K, Maruta A, et al. A prognostic score with pretransplant serum ferritin and disease status predicts outcome following reduced-intensity SCT. Bone Marrow Transplant. 2012;47(4):596-7.
83. Tachibana T, Tanaka M, Yamazaki E, Numata A, Takasaki H, Kuwabara H, et al. Multicenter validation of scoring system of pre-transplant serum ferritin and disease risk in patients with acute myeloid leukemia and myelodysplastic syndrome after allogeneic hematopoietic stem cell transplant. Leuk Lymphoma. 2013;54(6):1318-20.
84. Tachibana T, Takasaki H, Tanaka M, Maruta A, Hyo R, Ishigatsubo Y, et al. Serum ferritin and disease status at transplantation predict the outcome of allo-SCT in patients with AML or myelodysplastic syndrome. Bone Marrow Transplant. 2011;46(1):150-1.
85. Tachibana T, Tanaka M, Takasaki H, Numata A, Ito S, Watanabe R, et al. Pretransplant serum ferritin is associated with bloodstream infections within 100 days of allogeneic stem cell transplantation for myeloid malignancies. Int J Hematol. 2011;93(3):368-74.
86. Konuma T, Kato S, Oiwa-Monna M, Tojo A, Takahashi S. Pretransplant hyperferritinemia has no effect on the outcome of myeloablative cord blood transplantation for acute leukemia and myelodysplastic syndrome. Ann Hematol. 2014;93(6):1071-2.
87. Jang JE, Kim SJ, Cheong JW, Hyun SY, Kim YD, Kim YR, et al. Early CMV replication and subsequent chronic GVHD have a significant anti-leukemic effect after allogeneic HSCT in acute myeloid leukemia. Ann Hematol. 2015;94(2):275-82.
88. Sivgin S, Nazlim S, Zararsiz G, Baspinar O, Kaynar L, Deniz K, et al. Increased Bone Marrow Iron Scores Are Strongly Correlated With Elevated Serum Ferritin Levels and Poorer Survival in Patients With Iron Overload That Underwent Allogeneic Hematopoietic Stem Cell Transplantation: A Single Center Experience. Clin Lymphoma Myeloma Leuk. 2016;16(10):582-7.

89. Tachibana T, Tanaka M, Numata A, Matsumoto K, Tomita N, Fujimaki K, et al. Clinical significance of pre- and 1-year post-transplant serum ferritin among adult transplant recipients. Leuk Lymphoma. 2014;55(6):1350-6.
90. Cremers EM, van Biezen A, de Wreede LC, Scholten M, Vitek A, Finke J, et al. Prognostic pre-transplant factors in myelodysplastic syndromes primarily treated by high dose allogeneic hematopoietic stem cell transplantation: a retrospective study of the MDS subcommittee of the CMWP of the EBMT. Ann Hematol. 2016;95(12):1971-8.
91. Lebon D, Vergez F, Bertoli S, Harrivel V, De Botton S, Micol JB, et al. Hyperferritinemia at diagnosis predicts relapse and overall survival in younger AML patients with intermediate-risk cytogenetics. Leuk Res. 2015;39(8):818-21.
92. Tachibana T, Andou T, Tanaka M, Ito S, Miyazaki T, Ishii Y, et al. Clinical Significance of Serum Ferritin at Diagnosis in Patients With Acute Myeloid Leukemia: A YACHT Multicenter Retrospective Study. Clin Lymphoma Myeloma Leuk. 2018;18(6):415-21.
93. Cheng PP, Sun ZZ, Jiang F, Tang YT, Jiao XY. Hepcidin expression in patients with acute leukaemia. Eur J Clin Invest. 2012;42(5):517-25.
94. Aulbert E, Schmidt CG. Ferritin--a tumor marker in myeloid leukemia. Cancer Detect Prev. 1985;8(1-2):297-302.
95. Weber S, Parmon A, Kurrle N, Schnütgen F, Serve H. The Clinical Significance of Iron Overload and Iron Metabolism in Myelodysplastic Syndrome and Acute Myeloid Leukemia. Front Immunol. 2020;11:627662.
96. Gao M, Monian P, Pan Q, Zhang W, Xiang J, Jiang X. Ferroptosis is an autophagic cell death process. Cell Res. 2016;26(9):1021-32.
97. Zhang H, Sun C, Sun Q, Li Y, Zhou C. Susceptibility of acute myeloid leukemia cells to ferroptosis and evasion strategies. Front Mol Biosci. 2023;10:1275774.
98. Dorak MT. HFE H63D variant and leukemia susceptibility. Leuk Lymphoma. 2006;47(11):2269-70.
99. Viola A, Pagano L, Laudati D, D'Elia R, D'Amico MR, Ammirabile M, et al. HFE gene mutations in patients with acute leukemia. Leuk Lymphoma. 2006;47(11):2331-4.
100. Kennedy AE, Kamdar KY, Lupo PJ, Okcu MF, Scheurer ME, Baum MK, et al. Examination of HFE associations with childhood leukemia risk and extension to other iron regulatory genes. Leuk Res. 2014;38(9):1055-60.
101. Virtanen JM, Itälä-Remes MA, Remes KJ, Vahlberg T, Saunavaara JP, Sinisalo M, et al. Prognostic impact of pretransplant iron overload measured with magnetic resonance imaging on severe infections in allogeneic stem cell transplantation. Eur J Haematol. 2013;91(1):85-93.
102. Tamary H, Shalev H, Perez-Avraham G, Zoldan M, Levi I, Swinkels DW, et al. Elevated growth differentiation factor 15 expression in patients with congenital dyserythropoietic anemia type I. Blood. 2008;112(13):5241-4.
103. Finkenstedt A, Bianchi P, Theurl I, Vogel W, Witcher DR, Wroblewski VJ, et al. Regulation of iron metabolism through GDF15 and hepcidin in pyruvate kinase deficiency. Br J Haematol. 2009;144(5):789-93.
104. Finke J, Bethge WA, Schmoor C, Ottinger HD, Stelljes M, Zander AR, et al. Standard graft-versus-host disease prophylaxis with or without anti-T-cell globulin in haematopoietic cell transplantation from matched unrelated donors: a randomised, open-label, multicentre phase 3 trial. Lancet Oncol. 2009;10(9):855-64.
105. Socié G, Schmoor C, Bethge WA, Ottinger HD, Stelljes M, Zander AR, et al. Chronic graft-versus-host disease: long-term results from a randomized trial on graft-versus-host disease prophylaxis with or without anti-T-cell globulin ATG-Fresenius. Blood. 2011;117(23):6375-82.
106. Baron F, Fillet G, Beguin Y. Erythropoiesis after nonmyeloablative stem-cell transplantation is not impaired by inadequate erythropoietin production as observed after conventional allogeneic transplantation. Transplantation. 2002;74(12):1692-6.
107. Cox D. Sepsis - it is all about the platelets. Front Immunol. 2023;14:1210219.

108. Shragai T, Lebel E, Deshet D, Varon D, Avivi I, Kirgner I, et al. Characteristics and outcomes of adults with cytomegalovirus-associated thrombocytopenia: a case series and literature review. Br J Haematol. 2020;191(5):863-7.
109. Ritchie BM, Barreto JN, Barreto EF, Crow SA, Dierkhising RA, Jannetto PJ, et al. Relationship of Ganciclovir Therapeutic Drug Monitoring with Clinical Efficacy and Patient Safety. Antimicrob Agents Chemother. 2019;63(3).
110. Kato K, Kamezaki K, Shimoda K, Numata A, Haro T, Aoki K, et al. Intracellular signal transduction of interferon on the suppression of haematopoietic progenitor cell growth. Br J Haematol. 2003;123(3):528-35.
111. Hwang JH, Kim SW, Lee HJ, Yun HJ, Kim S, Jo DY. Interferon gamma has dual potential in inhibiting or promoting survival and growth of hematopoietic progenitors: interactions with stromal cell-derived factor 1. Int J Hematol. 2006;84(2):143-50.
112. Nishimoto M, Nakamae H, Koh H, Nakane T, Nakamae M, Hirose A, et al. Risk factors affecting cardiac left-ventricular hypertrophy and systolic and diastolic function in the chronic phase of allogeneic hematopoietic cell transplantation. Bone Marrow Transplant. 2013;48(4):581-6.
113. Hissen AH, Wan AN, Warwas ML, Pinto LJ, Moore MM. The Aspergillus fumigatus siderophore biosynthetic gene sidA, encoding L-ornithine N5-oxygenase, is required for virulence. Infect Immun. 2005;73(9):5493-503.
114. Sivgin S, Eser B. The management of iron overload in allogeneic hematopoietic stem cell transplant (alloHSCT) recipients: where do we stand? Ann Hematol. 2013;92(5):577-86.
115. Tamaki M, Nakasone H, Nakamura Y, Kawamura M, Kawamura S, Takeshita J, et al. Risk factors and outcomes of definite or clinical idiopathic pneumonia syndrome after allogeneic hematopoietic stem cell transplantation. Leuk Lymphoma. 2022;63(9):2197-205.
116. Recalcati S, Invernizzi P, Arosio P, Cairo G. New functions for an iron storage protein: the role of ferritin in immunity and autoimmunity. J Autoimmun. 2008;30(1-2):84-9.
117. Matzner Y, Hershko C, Polliack A, Konijn AM, Izak G. Suppressive effect of ferritin on in vitro lymphocyte function. Br J Haematol. 1979;42(3):345-53.
118. Cardier J, Romano E, Soyano A. Effect of hepatic isoferritins from iron overloaded rats on lymphocyte proliferative response: role of ferritin iron content. Immunopharmacol Immunotoxicol. 1995;17(4):719-32.
119. Broxmeyer HE, Mantel C, Gentile P, Srivastava C, Miyazawa K, Zucali JR, et al. Actions of H-subunit ferritin and lactoferrin as suppressor molecules of myelopoiesis in vitro and in vivo. Curr Stud Hematol Blood Transfus. 1991(58):178-81.
120. Gray CP, Arosio P, Hersey P. Heavy chain ferritin activates regulatory T cells by induction of changes in dendritic cells. Blood. 2002;99(9):3326-34.

# IV DISCUSSION AND PERSPECTIVES

## 1 Erythroferrone and hepcidin after allogeneic HSC transplantation

In the context of allo-HSCT, the significance of serum hepcidin and its interaction with iron metabolism and erythropoietic activity remained unexplored. Our team's initial investigations into hepcidin within the context of HSCT were conducted after auto-HSCT. The findings revealed that, despite a peak in hepcidin levels on the seventh day post-HCT, associations were observed with both erythropoietic activity and iron stores, underscoring their pivotal roles in hepcidin regulation, whereas inflammation exhibited only limited influence (1). Prior research into the erythropoietic regulator ERFE and its impact on iron mobilization and absorption through its inhibitory effect on hepcidin has paved the way for more extensive investigations (2). These discoveries spurred our study on the roles of ERFE and hepcidin in iron metabolism and erythropoiesis following allo-HSCT. This was facilitated by the availability of serum samples biobanked during a randomized trial of EPO therapy, which offered an additional opportunity to explore the interplay between iron, inflammation, and erythropoietic activity over time posttransplantation.

Following conditioning, a significant suppression of erythropoiesis was noted, with more pronounced effects observed following MA conditioning compared to NMA conditioning, impacting erythropoietic activity, Hb levels, and iron-related parameters. Our study unveiled a decline in ERFE levels in parallel with erythropoiesis suppression. The strong correlations between serum ERFE and erythropoietic markers (Hb, sTfR, reticulocytes) underscored the close relationship between erythropoietic activity and ERFE levels. Surprisingly, exogenous EPO administration did not lead to an anticipated increase in ERFE production (contrary to the significant sTfR response), in contrast to observations in healthy subjects. Our interpretation of this unexpected outcome is that part of the sTfR elevation is in fact more due to EPO-induced functional iron deficiency rather than stimulation of erythropoiesis. Furthermore, it is plausible that newly formed erythroid precursors, evolving within an altered environment, could exhibit an inappropriate capacity for ERFE secretion in response to EPO, or that unidentified inhibitory factors could play a role. To help sort that out, measurements of GDF-15 would help identify a potential component of ineffective erythropoiesis. In addition, the paracrine role of hepcidin, and particularly its impact on macrophages, warrants consideration. LysM-Atg5-/- mice, characterized by macrophage autophagy deficiency, exhibit a primary IO phenotype with high ferroportin expression in both macrophages and enterocytes (3). These findings suggest that macrophage autophagy restricts ferroportin expression and iron export, inducing hepcidin expression with an autocrine-paracrine effect that regulates ferroportin expression in duodenal enterocytes. This would necessitate more comprehensive research for elucidation.

While our study in a limited number of patients did not reveal a significant difference in ERFE during erythroid recovery between patients after MA and NMA conditioning, nor any correlation between CD34+ cell numbers and ERFE levels, more extensive investigations in a larger cohort could yield valuable insights into the potential influence of other factors such as graft source (BM, PBSC, or cord blood), donor characteristics (related or unrelated, matched or mismatched), conditioning (MA or NMA), or immunosuppressive regimen employed. Furthermore, the administration of post-transplant cyclophosphamide used in haploidentical allo-HSCT could potentially alter iron metabolism. Indeed, cyclophosphamide has been shown to induce iron accumulation in the liver and spleen of mice following suppression of erythropoiesis, upregulating hepcidin which further restricts iron availability for hemoglobin synthesis (4). However, precisely assessing its impact in the context of haplo-identical HSCT poses methodological challenges, notably due to the short time frame of only a few days between conditioning and cyclophosphamide administration. This temporal constraint would make it difficult to accurately isolate the changes in iron metabolism induced by cyclophosphamide itself. This comprehensive exploration would aim at elucidating the intricate factors that may contribute to the modulation of ERFE levels within the context of allo-HSCT.

Additionally, pretransplant hepcidin levels were closely linked to RBC transfusion history and SF levels, then displaying fluctuations corresponding to engraftment and subsequent erythropoietic recovery. Unlike the dynamics observed in thalassemia or auto-HSCT where the negative erythropoietic regulator prevail, allo-HSCT represents a distinct scenario where the impact of IO seems to surpass the typical control exerted by erythropoiesis on hepcidin production. Notably, individuals with high initial SF levels exhibited distinct responses to EPO therapy compared to those with lower levels. This observation highlights the intricate interplay between the relative responsiveness of iron burden and erythropoiesis. These findings open intriguing research prospects for the future, particularly in optimizing EPO therapy. As our group previous reported, significant disruptions occur in erythropoiesis and iron metabolism after allo-HSCT, characterized notably by inadequate endogenous EPO production. We demonstrated the efficacy and safety of using EPO during allo-HSCT, although not all patients exhibited a favorable response (5). It is plausible that a developing marrow could exhibit insufficient ERFE production in response to EPO due to its relatively early developmental state. ERFE could therefore be investigated as a predictive marker for EPO response. Insufficient ERFE levels could potentially indicate issues with erythropoietic reconstitution. Furthermore, we demonstrated that individuals with elevated pretransplant SF levels might not experience significant reduction in hepcidin levels in response to EPO treatment due to the more pronounced inducing effect of iron on hepcidin, impeding

mobilization of iron reserves for demanding erythroblasts. Consequently, SF levels could emerge as an additional crucial factor, alongside ERFE levels, for selecting candidates likely to optimally respond to EPO therapy in the context of allogeneic transplantation. However, given the exquisite response to EPO therapy observed after allo-HSCT (5-7), a large cohort of patients will be necessary to comprehensively investigate the underlying factors that predict this response, encompassing entities such as endogenous EPO, ERFE, hepcidin, ferritin, sTfR and other erythropoiesis- and iron-related parameters alongside standard blood counts, inflammation markers and clinical characteristics of the patient, donor and transplantation.

As illustrated in **Figure 1**, the underlying mechanisms of altered erythropoiesis and iron metabolism in various pathologies are increasingly understood (8). Schematically, two types of alterations, as well as their potential therapeutic targets, can be represented:

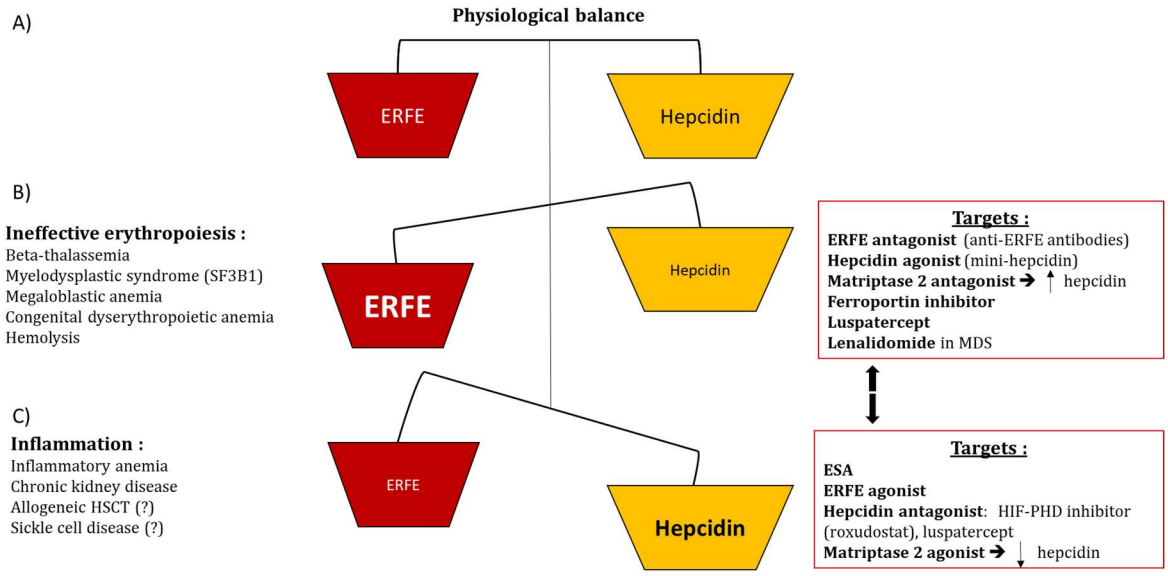


***Figure 1 : Altered interactions between erythropoiesis and iron metabolism in various pathological conditions.*** *A) Balance between ERFE and hepcidin production under normal physiological conditions. B) Pathologies associated with ineffective erythropoiesis, characterized by an excess of ERFE leading to inhibition of hepcidin. Several therapeutic targets have been described to either inhibit excess ERFE or stimulate the action or production of hepcidin. C) Pathologies characterized by ERFE deficiency and hepcidin overproduction. Various therapeutic targets are described to increase ERFE activity or inhibit the action or production of hepcidin.*

1. **Ineffective erythropoiesis**: ERFE excess and hepcidin insufficiency despite the inducing effect of IO (9). In beta-thalassemia, ineffective erythropoiesis (confirmed by elevated sTfR and GDF-15) leads to excessive production of ERFE, inducing hepcidin inhibition facilitating iron absorption by the intestine and release by macrophages, thereby contributing to IO already caused by transfusions (10, 11). This is also seen in MDS and in particular in sideroblastic

anemia, which has the lowest hepcidin/ferritin ratio, suggesting hepcidin insufficiency relative to IO. (12). Sideroblastic anemia is usually associated with a mutation in the splicing factor gene SF3B1, which leads to the expression of an alternative ERFE transcript that suppresses hepcidin transcription and so induces IO (13). Expression of the variant ERFE transcript was restricted to SF3B1-mutated erythroblasts and decreased in lenalidomide-responsive patients (13). In congenital dyserythropoietic anemia type II, a group of hereditary anemias characterized by dyserythropoiesis, IO, even in non-transfused patients, is also generated by ineffective erythropoiesis (confirmed by increased ERFE) (14).

2. **Inflammation**: hepcidin overproduction and ERFE deficiency. In ACD, there is an excess of hepcidin induced by IL-6, leading to functional iron deficiency. Additionally, as in chronic kidney disease, there is inadequate EPO production, hemodilution and direct inhibition of erythroid precursors by IFNs (8). SCD is a complex hemoglobinopathy characterized by less prominent ineffective erythropoiesis than in beta-thalassemia, less frequent need for transfusions, and a certain degree of inflammation, complicating the assessment of IO and the study of the ERFE-hepcidin axis (15, 16). Vaso-occlusive events induce inflammation, stimulating hepcidin production via the IL-6 pathway, while ineffective erythropoiesis increases GDF-15 and ERFE levels. However, hepcidin is not always decreased, and a loss of correlation between ERFE and hepcidin suggests that iron-inducing mechanisms may outweigh ERFE-mediated inhibition by erythropoiesis (17). The inclusion of SCD patients could provide interesting insights into alterations in iron metabolism associated with this pathology, but we could include only 2 such patients in our thesis.

In the context of allo-HSCT, increased hepcidin production despite post-transplant recovery of erythropoiesis is induced by inflammation and by IO secondary to transfusions and post-conditioning aplasia (**Figure 2)**. Measuring pro-inflammatory cytokines such as IL-6, IFN-γ, and TGF-β could assist in quantifying such inflammation and substantiating its involvement.

Comprehending how hepcidin and ERFE influence iron metabolism and erythropoiesis after allo-HSCT could pave the way for targeted therapeutic developments. Interventions aiming at specifically modulating hepcidin or ERFE levels could assist in managing posttransplant iron metabolism-related dysfunctions (**Figure 2**). For example, in animal models of β-thalassemia, administration of hepcidin (18), a ferroportin inhibitor (19) or a matriptase antagonist (20) has shown variable improvements of ineffective erythropoiesis and IO. Moreover, Arezes *et al.* introduced a monoclonal antibody against ERFE to prevent hepcidin suppression by erythropoiesis. Through experimentation on a mouse model of β-thalassemia intermedia, the study revealed that the monoclonal antibody raised hemoglobin levels and concurrently decreased serum iron concentration, effectively addressing systemic iron overload (21).

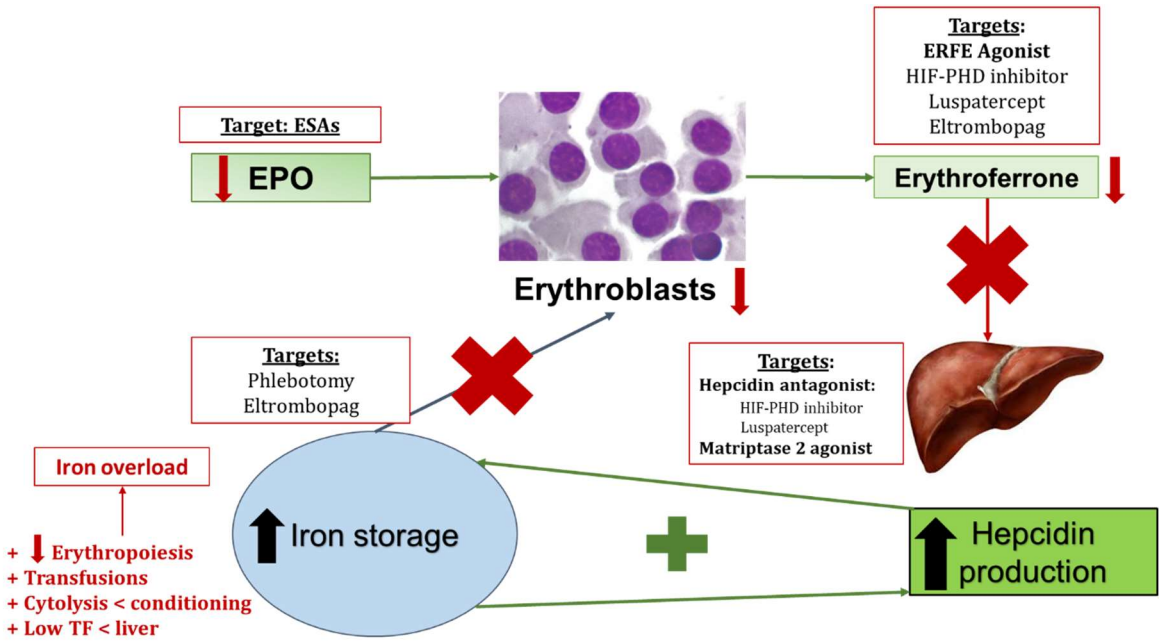


***Figure 2*: Hypothetical dynamics of iron regulation by erythropoiesis during allo-HSCT and potential therapeutic targets.** *After transplantation, decreased production of endogenous EPO is often observed. Although ERFE is secreted, inhibition of hepcidin production appears to be counteracted by the effect of liver IO. Iron stores are increased by hepatic cytolysis due to conditioning, transfusions, decreased transferrin production and aplasia (which limits iron utilization). Several therapeutic approaches can be considered to stimulate erythropoiesis and reduce hepcidin, including ESAs, ERFE agonists, HIF-PDH inhibitors that increase ERFE production while inhibiting hepcidin, eltrombopag which acts on erythroid differentiation and reduces iron stores, hepcidin antagonists and matriptase agonists. When iron overload is significant and erythroid function is restored (typically 6 to 12 months after transplantation), phlebotomies remain an easy therapeutic option to counter iron overload.*

Likewise, employing a monoclonal antibody against hepcidin to mitigate its excess warrants consideration. Disrupted iron homeostasis due to hepcidin overexpression has been associated with ACD. This perturbation leads to impaired erythropoiesis and reduced responsiveness to ESAs (22). Consequently, targeting the suppression of hepcidin has emerged as a potential therapeutic approach for addressing ACD. Cooke *et al.* directed their efforts towards developing fully human anti-hepcidin antibodies with potential application in treating ACD and other disorders related to iron distribution (23). Using mice bearing the human hepcidin gene, these antibodies were shown to increase serum iron levels, promoting enhanced hemoglobinization of RBCs. One specific antibody, 12B9m, was validated in a mouse model of ACD and demonstrated the capacity to modulate serum iron levels in cynomolgus monkeys. The 12B9m antibody presents a promising avenue as a prospective therapeutic option for addressing ACD, particularly in patients with kidney disease or cancer, and may hold potential utility in the context of allo-HSCT as well. Finally, Roxadustat, an oral hypoxia-inducible factor prolyl hydroxylase (HIF-PHD) inhibitor induces a transient "pseudo-hypoxic" state mimicking cellular conditions for EPO production and enhances iron availability by reducing hepcidin levels, effectively addressing functional iron deficiency observed in resistance to conventional

ESAs (24, 25). The reduction in hepcidin levels is more pronounced with Roxadustat than with ESAs, making it a unique molecule that enhances erythropoiesis while bypassing iron metabolism disorders. While its impact on ERFE has not yet been elucidated, it is linked to a more pronounced reduction in SF levels and an increase in SeFe levels compared to a decrease of both parameters with ESAs, suggesting that it promotes the release of sequestered storage iron for incorporation into erythroid progenitors (25). However, Vadadustat, another HIF-PHD inhibitor, demonstrates ERFE-independent effects on erythropoiesis and iron metabolism, normalizing Hb and modulating iron parameters in both wild type and ERFE knockout CKD mouse models (26). This observation suggests that hepcidin inhibition occurs directly through the effect of HIF, but this remains to be investigated in other models. Studies of HIF-PHD inhibitors in chronic kidney disease have demonstrated comparable efficacy with less requirement for intravenous iron supplementation compared to ESAs (25, 27, 28). Exploring their effectiveness in other contexts of anemia, such as ACD, would be relevant. Finally, Eltrombopag, a TPO receptor agonist primarily used in immune thrombocytopenia, has shown efficacy in enhancing erythroid differentiation (29) and in restoring the three cell lineages in aplastic anemia (30). Recently, studies have highlighted a significant role of Eltrombopag as iron chelator (31), achieving this effect both directly by binding to iron and indirectly by regulating iron outflow (32).

This multidimensional approach, accounting for the complex interactions between hepcidin, ERFE, and iron burden, could potentially enhance the precision and efficacy of therapeutic strategies in the context of allo-HSCT. Moreover, the incorporation of ERFE and hepcidin measurements holds significant promise in understanding erythropoiesis and iron metabolism beyond allo-HSCT, in conditions such as MDS or MPN and even chronic obstructive pulmonary disease (COPD). Specifically, hepcidin is elevated in patients with mild-to-moderate COPD or during an exacerbation event, but reduced in patients with advanced and severe COPD (correlating with hypoxemia), suggesting that the effect of hypoxia dominates that of inflammation on hepcidin production (33, 34). In a murine COPD model, cigarette smoke exposure decreased lung and circulating hepcidin levels and increased ferroportin expression on alveolar macrophages, while blood EPO and ERFE levels were increased (35). However, alveolar macrophages failed to increase hepcidin in response to bacterial infection, impairing functional responses to Streptococcus pneumoniae infection (35).

## 2 Pretransplant serum ferritin and hepcidin and outcomes after allogeneic hematopoietic stem cell transplantation

The influence of SF and hepcidin on outcomes following allo-HSCT has yielded conflicting findings, sparking debate. SF has been employed as a surrogate marker for IO, yet its interpretation is complicated by factors such as inflammation and liver disease. Studies quantifying IO by MRI prior to transplantation were combined in a meta-analysis indicating no significant effect of liver iron concentrations on survival or complications after allo-HSCT, but individual studies were less clearcut as half of them pointed to adverse outcomes with IO. Meanwhile, the role of hepcidin in iron regulation prompted investigations into its association with complications post allo-HSCT, but results have also been contradictory.

Our study aimed to assess the impact of pre-conditioning SF and hepcidin levels on outcomes in a cohort of 502 allo-HSCT patients. Through our investigation, we unveiled a compelling positive correlation between pretransplant hepcidin and SF levels. This correlation highlights the intricate interplay between iron metabolism markers and their potential interaction in predicting patient outcomes. Furthermore, our analysis delved deeper into the determinants of hepcidin levels, revealing their susceptibility to multiple factors including pretransplant RBC transfusions, inflammatory processes, comorbidities, performance status, and low pre-conditioning neutrophils. Meanwhile, SF levels themselves were found to be significantly influenced by a complex interplay of various determinants, encompassing pre-conditioning transferrin saturation, RBC and platelet transfusions, anemia, liver and kidney function, type of disease (high SF in AL), performance status and several other patient characteristics. This intertwined relationship between hepcidin and SF levels indicates a dynamic and multifaceted iron regulatory mechanism, responsive to both exogenous factors like RBC transfusions and endogenous factors such as inflammation and patient characteristics. These findings extend beyond a broad association and emphasize the intricate interdependence between these iron-related biomarkers. Consequently, these primary results demonstrate that SF does not solely represent iron burden but also reflects a series of other detrimental clinical characteristics of patients. Furthermore, hepcidin levels are themselves influenced by factors such as the HCT-CI score and performance status, both of which are pejorative parameters that can impact clinical outcomes.

Several studies have highlighted the presence of polymorphisms in the HFE gene among patients with hematologic disorders, mainly acute leukemias (36-38). These polymorphisms predispose individuals to IO due to insufficient hepcidin production under normal physiological conditions, independently of external factors such as transfusions or inflammation. Furthermore, an association between HFE gene mutations, IO, and cancer development has been established (39). This association is reinforced in the presence of

variants in the transferrin receptor gene, underscoring their biological interaction in iron transfer across membranes (40). Additionally, leukemic blasts express high levels of transferrin receptors (41, 42). Exploring HFE genetics within our cohort of 502 patients and examining whether carriers of HFE mutations cluster in the fourth quartile of ferritin levels would be quite interesting. Subsequently, investigating the implications of this polymorphism on survival outcomes, particularly in terms of relapse, would be worthwhile.

We highlighted the predictive value of elevated pre-conditioning SF levels, associating them with unfavorable outcomes in univariate analysis, such as a decrease in OS and PFS, an increase in NRM, infections and sepsis, as well as specific toxicities like gastrointestinal toxicity. Additionally, elevated SF correlated with a prolonged recovery time of all three blood lineages, a longer time to achieve RBC and platelet transfusion independence, and an increased number of posttransplant transfusions. Hepcidin levels displayed a weaker impact on outcomes compared to SF levels and were not significant in multivariate models, except for conditioning-related skin toxicity. These results are summarized **in Table 1**.

***Table 1**: Summary of univariate and multivariate analyses of the impact of pretransplant hepcidin and ferritin on various outcomes.*

| | Univariate analysis | | | | Multivariate analysis | | | |
|---|---|---|---|---|---|---|---|---|
| **Post-transplant outcomes** | **Hepcidin** | | **Ferritin** | | **Hepcidin** | | **Ferritin** | |
| | **HR** | **P-value** | **HR** | **P-value** | **HR** | **P-value** | **HR** | **P-value** |
| **Overall survival** | 1.24 | **0.002** | 1.25 | **<0.0001** | **Not significant** | | **1.17** | **0.01** |
| **Progression-free survival** | 1.16 | **0.03** | 1.26 | **0.0002** | | | **1.19** | **0.02** |
| **Non-relapse mortality** | 1.14 | 0.13 | 1.21 | **0.02** | | | **Not significant** | |
| **Time to RBC Tx independence** | 0.89 | 0.06 | 0.90 | **0.03** | | | **Not significant** | |
| **Time to platelet Tx independence** | 0.88 | **0.04** | 0.87 | **0.007** | | | | |
| **Time to neutrophils ≥ 1 $10^9$/L** | 0.99 | 0.88 | 0.89 | **0.003** | | | **Not significant** | |
| **Time to platelets ≥ 50 $10^9$/L** | 0.90 | **0.04** | 0.84 | **0.0003** | | | | |
| **Time to hemoglobin ≥ 10 g/dl** | 0.91 | 0.10 | 0.90 | **0.02** | | | | |
| **Time to first infection** | 1.02 | 0.68 | 1.17 | **0.0003** | | | **Not significant** | |
| **Acute and chronic GVHD** | **Not significant** | | | | | | | |

*Tx :* transfusion.

However, SF was not confirmed as an independent predictor of these outcomes, as it lost significance in multivariate Cox models of several outcomes, and our findings rather revealed an intricate involvement of various factors (biological markers, patient comorbidities, and transplant characteristics) in shaping these outcomes. Pre-conditioning SF retained prognostic value in multivariate analysis for long-term OS, PFS at 100 days and 1-year, gastrointestinal toxicity, and the number of posttransplant RBC transfusions. Our comprehensive multivariate

analysis demonstrated that long-term survival was best predicted by a combination of pretransplant SF, DRI, performance status and age. Similarly, short-term or mild-term PFS (at 100 days and 1 year, respectively) were anticipated by pretransplant SF, total bilirubin, performance status and DRI together with other clinical factors. These observations underscore the potential of SF as an early indicator of high-risk patients and aligns with the prognostic score for OS and PFS proposed by Armand *et al.* (43), which incorporates pretransplant SF, age and DRI for patients with acute leukemia or MDS undergoing allo-HSCT. They are also consistent with the augmented HCT-CI proposed by Vaughn *et al.*, which combines the HCT-CI with age and 3 biomarkers (albumin, platelets and ferritin) to predict NRM after HLA-matched related donor allo-HSCT (44) as well as after alternative donor transplantation (45). Furthermore, low pre-transplant serum albumin was a significant predictor of NRM at 1 year in our multivariate model. The integration of SF, an easily accessible and cost-effective biomarker, into risk stratification and treatment optimization strategies, stands out as a promising avenue to enhance patient care. Large-scale retrospective analyses could validate the additional predictive value of SF in diverse transplantation scenarios (MA, NMA and RIC) and diseases. These endeavors would materialize the ability for personalized risk assessment and informed decision-making.

The potential to enhance outcomes after allo-HSCT using SF as a predictive marker may also justify the investigation of clinical interventions. Indeed, as IO is a significant factor contributing to elevated SF levels, prospective investigations could evaluate targeted interventions aimed at modulating iron metabolism prior to allo-HSCT. For instance, exploring whether preemptive reduction of IO through iron chelation or by more drastically adjusting transfusion thresholds (based on the patient's clinical tolerance) may lead to improved outcomes, even if these interventions take time to hold their promise. Essmann *et al.* examined the effect of iron chelation with deferasirox during conditioning in 25 patients undergoing hematopoietic stem cell transplantation. Elevated levels of LPI were associated with increased infection risks, but iron chelation with deferasirox effectively suppressed the appearance of LPI and was well-tolerated (46). The safety and impact of this chelation on transplant outcomes remains to be determined on a larger scale and with longer follow-up. The predictive role of SF could also open avenues for tailoring supportive care regimens. For patients with higher pre-conditioning SF levels, more aggressive preventive measures against infections and specific conditioning-related toxicities could be implemented. This personalized approach could enhance patient outcomes and minimize treatment-related complications.

Moreover, although the evidence for this remains largely controversial (47), several studies have demonstrated that iron could promote tumor growth and relapse (48, 49), and chelation therapy targeting iron has shown potential in limiting this effect (50). However, few studies

have found an influence of pretransplant SF on the risk of relapse in multivariate models (51-55), as contradictory outcomes emerge (55) and the majority of other investigations indicated the absence of a correlation between SF levels and relapse after transplantation except in AL patients (55-65). We did not observe a significant difference in relapse based on pretransplant SF levels across the entire cohort. However, relapse was significantly more frequent among AL patients when pretransplant SF levels were elevated. It is noteworthy that elevated levels of GDF-15 have shown an increase during AL relapses, suggesting its potential relevance as a biological marker post-transplantation (66). Thus, the impact of pretransplant SF and hepcidin on relapse should be studied within patient subgroups (selected based on their initial disease and conditioning regimen) rather than the entire cohort where heterogeneity is too important. Therefore, we should examine homogeneous patient groups (e.g., multiple myeloma treated with high-dose melphalan and autologous HSCT, lymphomas treated with the BEAM regimen and autologous transplantation, acute leukemias treated with cyclophosphamide and TBI or low-dose TBI and fludarabine followed by allo-HSCT) and analyze the relationship between pre- and posttransplant iron status and the incidence of relapse, considering the usual cofactors associated with relapse risk. Additionally, exploring the controversial connection between iron metabolism and cancer relapse has implications not only for post-transplant care but also for broader oncological contexts. The potential for iron chelation therapy to modulate tumor growth raises intriguing possibilities for adjunctive treatments that could complement existing cancer therapies. By analyzing well-defined patient groups undergoing different transplantation procedures, we aim to elucidate whether there is a consistent pattern between iron status and relapse incidence across various transplantation contexts. This research could provide valuable evidence for the consideration of iron modulation strategies in the management of both post-transplant complications and cancer recurrence. Finally, as demonstrated in our study, the immediate post-transplantation period is characterized by a disproportionate increase in SF levels. However, the reasons for this phenomenon remain unexplored. Within multivariate models, we plan to analyze the determinants of SF, SeFe and Tsat at various post-transplantation time points, extending up to 10 years post-transplant. This study would aim to uncover factors influencing iron parameters beyond iron stores, by examining a range of clinical and biological factors relevant to these biomarkers. Exploring the potential impact of post-transplant cyclophosphamide would be challenging due to the short interval between conditioning and cyclophosphamide administration that could prevent an appropriate separation of their respective effect. This would also be done with the intention of addressing the question: at what point can serum ferritin levels be reconsidered as an accurate quantitative measure of iron status? And a correlate of this would be: at which point and with

which endpoint should we intervene with iron-removing strategies such as phlebotomies and chelation to prevent the long-term damages of persisting iron overload.

In conclusion, our study highlights the significance of SF as a predictive biomarker for allo-HSCT outcomes. The substantial influence of SF, surpassing that of hepcidin, provides a foundation for future research. With its ease of measurement and clinical feasibility, SF emerges as a valuable tool for assessing the risk of complications following allo-HSCT. Furthermore, our findings emphasize the importance of considering iron metabolism in therapeutic interventions (e.g., chelation), opening new avenues to enhance allo-HSCT outcomes. This comprehensive understanding of the intricate interplay of iron-related biomarkers offers a more refined approach to patient care and therapeutic strategies in the context of allo-HSCT.

# 3 References:


1. Jaspers A, Baron F, Willems E, Seidel L, Wiegerinck ET, Swinkels DW, et al. Serum hepcidin following autologous hematopoietic cell transplantation: an illustration of the interplay of iron status, erythropoiesis and inflammation. Haematologica. 2014;99(3):e35-7.
2. Kautz L, Jung G, Valore EV, Rivella S, Nemeth E, Ganz T. Identification of erythroferrone as an erythroid regulator of iron metabolism. Nat Genet. 2014;46(7):678-84.
3. Taleb M, Maillet I, Le Bert M, Mura C. Targeted autophagy disruption reveals the central role of macrophage iron metabolism in systemic iron homeostasis. Blood. 2022;140(4):374-87.
4. Sheng Y, Chen YJ, Qian ZM, Zheng J, Liu Y. Cyclophosphamide induces a significant increase in iron content in the liver and spleen of mice. Hum Exp Toxicol. 2020;39(7):973-83.
5. Jaspers A, Baron F, Willems E, Seidel L, Hafraoui K, Vanstraelen G, et al. Erythropoietin therapy after allogeneic hematopoietic cell transplantation: a prospective, randomized trial. Blood. 2014;124(1):33-41.
6. Baron F, Sautois B, Baudoux E, Matus G, Fillet G, Beguin Y. Optimization of recombinant human erythropoietin therapy after allogeneic hematopoietic stem cell transplantation. Exp Hematol. 2002;30(6):546-54.
7. Vanstraelen G, Baron F, Willems E, Bonnet C, Hafraoui K, Frère P, et al. Recombinant human erythropoietin therapy after allogeneic hematopoietic cell transplantation with a nonmyeloablative conditioning regimen: low donor chimerism predicts for poor response. Exp Hematol. 2006;34(7):841-50.
8. Camaschella C, Nai A, Silvestri L. Iron metabolism and iron disorders revisited in the hepcidin era. Haematologica. 2020;105(2):260-72.
9. Cazzola M. Ineffective erythropoiesis and its treatment. Blood. 2022;139(16):2460-70.
10. Taher AT, Weatherall DJ, Cappellini MD. Thalassaemia. Lancet. 2018;391(10116):155-67.
11. Huang Y, Lei Y, Liu R, Liu J, Yang G, Xiang Z, et al. Imbalance of erythropoiesis and iron metabolism in patients with thalassemia. Int J Med Sci. 2019;16(2):302-10.
12. Santini V, Girelli D, Sanna A, Martinelli N, Duca L, Campostrini N, et al. Hepcidin levels and their determinants in different types of myelodysplastic syndromes. PLoS One. 2011;6(8):e23109.
13. Bondu S, Alary AS, Lefèvre C, Houy A, Jung G, Lefebvre T, et al. A variant erythroferrone disrupts iron homeostasis in SF3B1-mutated myelodysplastic syndrome. Sci Transl Med. 2019;11(500).
14. Russo R, Andolfo I, Manna F, De Rosa G, De Falco L, Gambale A, et al. Increased levels of ERFE-encoding *FAM132B* in patients with congenital dyserythropoietic anemia type II. Blood. 2016;128(14):1899-902.
15. Girelli D, Busti F. ERFE regulation in sickle cell disease: complex but promising. Br J Haematol. 2020;189(6):1012-3.
16. van Vuren AJ, Sharfo A, Grootendorst ST, van Straaten S, Punt AM, Petersen JB, et al. A Comprehensive Analysis of the Erythropoietin-erythroferrone-hepcidin pathway in hereditary hemolytic anemias. Hemasphere. 2021;5(9):e627.
17. Mangaonkar AA, Thawer F, Son J, Ajebo G, Xu H, Barrett NJ, et al. Regulation of iron homeostasis through the erythroferrone-hepcidin axis in sickle cell disease. Br J Haematol. 2020;189(6):1204-9.
18. Casu C, Oikonomidou PR, Chen H, Nandi V, Ginzburg Y, Prasad P, et al. Minihepcidin peptides as disease modifiers in mice affected by β-thalassemia and polycythemia vera. Blood. 2016;128(2):265-76.
19. Manolova V, Nyffenegger N, Flace A, Altermatt P, Varol A, Doucerain C, et al. Oral ferroportin inhibitor ameliorates ineffective erythropoiesis in a model of β-thalassemia. J Clin Invest. 2019;130(1):491-506.
20. Guo S, Casu C, Gardenghi S, Booten S, Aghajan M, Peralta R, et al. Reducing TMPRSS6 ameliorates hemochromatosis and β-thalassemia in mice. J Clin Invest. 2013;123(4):1531-41.

21. Arezes J, Foy N, McHugh K, Quinkert D, Benard S, Sawant A, et al. Antibodies against the erythroferrone N-terminal domain prevent hepcidin suppression and ameliorate murine thalassemia. Blood. 2020;135(8):547-57.
22. Hörl WH, Jacobs C, Macdougall IC, Valderrábano F, Parrondo I, Thompson K, et al. European best practice guidelines 14-16: inadequate response to epoetin. Nephrol Dial Transplant. 2000;15 Suppl 4:43-50.
23. Cooke KS, Hinkle B, Salimi-Moosavi H, Foltz I, King C, Rathanaswami P, et al. A fully human anti-hepcidin antibody modulates iron metabolism in both mice and nonhuman primates. Blood. 2013;122(17):3054-61.
24. Locatelli F, Fishbane S, Block GA, Macdougall IC. Targeting Hypoxia-Inducible Factors for the Treatment of Anemia in Chronic Kidney Disease Patients. Am J Nephrol. 2017;45(3):187-99.
25. Ganz T, Locatelli F, Arici M, Akizawa T, Reusch M. Iron Parameters in Patients Treated with Roxadustat for Anemia of Chronic Kidney Disease. J Clin Med. 2023;12(13).
26. Hanudel MR, Wong S, Jung G, Qiao B, Gabayan V, Zuk A, et al. Amelioration of chronic kidney disease-associated anemia by vadadustat in mice is not dependent on erythroferrone. Kidney Int. 2021;100(1):79-89.
27. Yang J, Xing J, Zhu X, Xie X, Wang L, Zhang X. Effects of hypoxia-inducible factor-prolyl hydroxylase inhibitors. Front Endocrinol (Lausanne). 2023;14:1131516.
28. Sugahara M, Tanaka T, Nangaku M. Prolyl hydroxylase domain inhibitors as a novel therapeutic approach against anemia in chronic kidney disease. Kidney Int. 2017;92(2):306-12.
29. Qanash H, Li Y, Smith RH, Linask K, Young-Baird S, Hakami W, et al. Eltrombopag Improves Erythroid Differentiation in a Human Induced Pluripotent Stem Cell Model of Diamond Blackfan Anemia. Cells. 2021;10(4).
30. Desmond R, Townsley DM, Dumitriu B, Olnes MJ, Scheinberg P, Bevans M, et al. Eltrombopag restores trilineage hematopoiesis in refractory severe aplastic anemia that can be sustained on discontinuation of drug. Blood. 2014;123(12):1818-25.
31. Vlachodimitropoulou E, Chen YL, Garbowski M, Koonyosying P, Psaila B, Sola-Visner M, et al. Eltrombopag: a powerful chelator of cellular or extracellular iron(III) alone or combined with a second chelator. Blood. 2017;130(17):1923-33.
32. Di Paola A, Tortora C, Argenziano M, Marrapodi MM, Rossi F. Emerging Roles of the Iron Chelators in Inflammation. Int J Mol Sci. 2022;23(14).
33. Duru S, Bilgin E, Ardiç S. Hepcidin: A useful marker in chronic obstructive pulmonary disease. Ann Thorac Med. 2012;7(1):31-5.
34. Nickol AH, Frise MC, Cheng HY, McGahey A, McFadyen BM, Harris-Wright T, et al. A cross-sectional study of the prevalence and associations of iron deficiency in a cohort of patients with chronic obstructive pulmonary disease. BMJ Open. 2015;5(7):e007911.
35. Perez E, Baker JR, Di Giandomenico S, Kermani P, Parker J, Kim K, et al. Hepcidin Is Essential for Alveolar Macrophage Function and Is Disrupted by Smoke in a Murine Chronic Obstructive Pulmonary Disease Model. J Immunol. 2020;205(9):2489-98.
36. Kennedy AE, Kamdar KY, Lupo PJ, Okcu MF, Scheurer ME, Baum MK, et al. Examination of HFE associations with childhood leukemia risk and extension to other iron regulatory genes. Leuk Res. 2014;38(9):1055-60.
37. Dorak MT. HFE H63D variant and leukemia susceptibility. Leuk Lymphoma. 2006;47(11):2269-70.
38. Viola A, Pagano L, Laudati D, D'Elia R, D'Amico MR, Ammirabile M, et al. HFE gene mutations in patients with acute leukemia. Leuk Lymphoma. 2006;47(11):2331-4.
39. Beckman LE, Van Landeghem GF, Sikström C, Wahlin A, Markevärn B, Hallmans G, et al. Interaction between haemochromatosis and transferrin receptor genes in different neoplastic disorders. Carcinogenesis. 1999;20(7):1231-3.
40. Benyamin B, McRae AF, Zhu G, Gordon S, Henders AK, Palotie A, et al. Variants in TF and HFE explain approximately 40% of genetic variation in serum-transferrin levels. Am J Hum Genet. 2009;84(1):60-5.

41. Yeh CJ, Taylor CG, Faulk WP. Transferrin binding by peripheral blood mononuclear cells in human lymphomas, myelomas and leukemias. Vox Sang. 1984;46(4):217-23.
42. Lyons VJ, Pappas D. Affinity separation and subsequent terminal differentiation of acute myeloid leukemia cells using the human transferrin receptor (CD71) as a capture target. Analyst. 2019;144(10):3369-80.
43. Armand P, Kim HT, Cutler CS, Ho VT, Koreth J, Ritz J, et al. A prognostic score for patients with acute leukemia or myelodysplastic syndromes undergoing allogeneic stem cell transplantation. Biol Blood Marrow Transplant. 2008;14(1):28-35.
44. Vaughn JE, Storer BE, Armand P, Raimondi R, Gibson C, Rambaldi A, et al. Design and Validation of an Augmented Hematopoietic Cell Transplantation-Comorbidity Index Comprising Pretransplant Ferritin, Albumin, and Platelet Count for Prediction of Outcomes after Allogeneic Transplantation. Biol Blood Marrow Transplant. 2015;21(8):1418-24.
45. Elsawy M, Storer BE, Milano F, Sandmaier BM, Delaney C, Salit RB, et al. Prognostic Performance of the Augmented Hematopoietic Cell Transplantation-Specific Comorbidity/Age Index in Recipients of Allogeneic Hematopoietic Stem Cell Transplantation from Alternative Graft Sources. Biol Blood Marrow Transplant. 2019;25(5):1045-52.
46. Essmann S, Heestermans M, Dadkhah A, Janson D, Wolschke C, Ayuk F, et al. Iron Chelation with Deferasirox Suppresses the Appearance of Labile Plasma Iron During Conditioning Chemotherapy Prior to Allogeneic Stem Cell Transplantation. Transplant Cell Ther. 2023;29(1):42.e1-.e6.
47. Beguin Y, Aapro M, Ludwig H, Mizzen L, Osterborg A. Epidemiological and nonclinical studies investigating effects of iron in carcinogenesis--a critical review. Crit Rev Oncol Hematol. 2014;89(1):1-15.
48. Hassannia B, Vandenabeele P, Vanden Berghe T. Targeting Ferroptosis to Iron Out Cancer. Cancer Cell. 2019;35(6):830-49.
49. Battaglia AM, Chirillo R, Aversa I, Sacco A, Costanzo F, Biamonte F. Ferroptosis and Cancer: Mitochondria Meet the "Iron Maiden" Cell Death. Cells. 2020;9(6).
50. Chen Y, Ohara T, Xing B, Qi J, Noma K, Matsukawa A. A Promising New Anti-Cancer Strategy: Iron Chelators Targeting CSCs. Acta Med Okayama. 2020;74(1):1-6.
51. Duda K, Wieczorkiewicz-Kabut A, Spałek A, Koclęga A, Kopińska AJ, Woźniczka K, et al. Room for Improvement: A 20-Year Single Center Experience with Allogeneic Stem Cell Transplantation for Myelodysplastic Syndromes. Indian J Hematol Blood Transfus. 2022;38(4):680-90.
52. Tachibana T, Tanaka M, Numata A, Takasaki H, Ito S, Ohshima R, et al. Pretransplant serum ferritin has a prognostic influence on allogeneic transplant regardless of disease risk. Leuk Lymphoma. 2012;53(3):456-61.
53. Kim YR, Kim JS, Cheong JW, Song JW, Min YH. Transfusion-associated iron overload as an adverse risk factor for transplantation outcome in patients undergoing reduced-intensity stem cell transplantation for myeloid malignancies. Acta Haematol. 2008;120(3):182-9.
54. Wahlin A, Lorenz F, Fredriksson M, Remberger M, Wahlin BE, Hägglund H. Hyperferritinemia is associated with low incidence of graft versus host disease, high relapse rate, and impaired survival in patients with blood disorders receiving allogeneic hematopoietic stem cell grafts. Med Oncol. 2011;28(2):552-8.
55. Jang JE, Kim SJ, Cheong JW, Hyun SY, Kim YD, Kim YR, et al. Early CMV replication and subsequent chronic GVHD have a significant anti-leukemic effect after allogeneic HSCT in acute myeloid leukemia. Ann Hematol. 2015;94(2):275-82.
56. Oakes R, Sood N, Pearce R, Swirsky D, Cook G, Gilleece M. Prognostic impact of serum ferritin concentration on survival following reduced-intensity conditioned allogeneic haemopoietic SCT. Bone Marrow Transplant. 2010;45(12):1754-5.
57. Akı Ş, Suyanı E, Bildacı Y, Çakar MK, Baysal NA, Sucak GT. Prognostic role of pre-transplantation serum C-reactive protein levels in patients with acute leukemia undergoing myeloablative allogeneic stem cell transplantation. Clin Transplant. 2012;26(5):E513-21.

58. Tanaka M, Kanamori H, Matsumoto K, Tachibana T, Numata A, Ohashi K, et al. Clinical significance of pretransplant serum ferritin on the outcome of allogeneic hematopoietic SCT: a prospective cohort study by the Kanto Study Group for Cell Therapy. Bone Marrow Transplant. 2015;50(5):727-33.
59. Cremers EM, van Biezen A, de Wreede LC, Scholten M, Vitek A, Finke J, et al. Prognostic pre-transplant factors in myelodysplastic syndromes primarily treated by high dose allogeneic hematopoietic stem cell transplantation: a retrospective study of the MDS subcommittee of the CMWP of the EBMT. Ann Hematol. 2016;95(12):1971-8.
60. Sivgin S, Nazlim S, Zararsiz G, Baspinar O, Kaynar L, Deniz K, et al. Increased Bone Marrow Iron Scores Are Strongly Correlated With Elevated Serum Ferritin Levels and Poorer Survival in Patients With Iron Overload That Underwent Allogeneic Hematopoietic Stem Cell Transplantation: A Single Center Experience. Clin Lymphoma Myeloma Leuk. 2016;16(10):582-7.
61. Sivgin S, Baldane S, Kaynar L, Kurnaz F, Pala C, Ozturk A, et al. Pretransplant serum ferritin level may be a predictive marker for outcomes in patients having undergone allogeneic hematopoietic stem cell transplantation. Neoplasma. 2012;59(2):183-90.
62. Mahindra A, Bolwell B, Sobecks R, Rybicki L, Pohlman B, Dean R, et al. Elevated pretransplant ferritin is associated with a lower incidence of chronic graft-versus-host disease and inferior survival after myeloablative allogeneic haematopoietic stem cell transplantation. Br J Haematol. 2009;146(3):310-6.
63. Artz AS, Logan B, Zhu X, Akpek G, Bufarull RM, Gupta V, et al. The prognostic value of serum C-reactive protein, ferritin, and albumin prior to allogeneic transplantation for acute myeloid leukemia and myelodysplastic syndromes. Haematologica. 2016;101(11):1426-33.
64. Sakamoto S, Kawabata H, Kanda J, Uchiyama T, Mizumoto C, Kitano T, et al. High pretransplant hepcidin levels are associated with poor overall survival and delayed platelet engraftment after allogeneic hematopoietic stem cell transplantation. Cancer Med. 2017;6(1):120-8.
65. Eisfeld AK, Westerman M, Krahl R, Leiblein S, Liebert UG, Hehme M, et al. Highly Elevated Serum Hepcidin in Patients with Acute Myeloid Leukemia prior to and after Allogeneic Hematopoietic Cell Transplantation: Does This Protect from Excessive Parenchymal Iron Loading? Adv Hematol. 2011;2011:491058.
66. Hegab HM, El-Ghammaz AMS, El-Razzaz MK, Helal RAA. Prognostic Impact of Serum Growth Differentiation Factor 15 Level in Acute Myeloid Leukemia Patients. Indian J Hematol Blood Transfus. 2021;37(1):37-44.