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Edited by Zaccheaus Awortu Jeremiah



Human Iron Metabolism - Biology, Health, and Disease

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Volume 34

Aims and Scope of the Series

Modern physiology requires a comprehensive understanding of the integration of tissues and organs throughout the mammalian body, including the cooperation between structure and function at the cellular and molecular levels governed by gene and protein expression. While a daunting task, learning is facilitated by identifying common and effective signaling pathways mediated by a variety of factors employed by nature to preserve and sustain homeostatic life. As a leading example, the cellular interaction between intracellular concentration of Ca+2 increases, and changes in plasma membrane potential is integral for coordinating blood flow, governing the exocytosis of neurotransmitters, and modulating gene expression and cell effector secretory functions. Furthermore, in this manner, understanding the systemic interaction between the cardiovascular and nervous systems has become more important than ever as human populations' life prolongation, aging and mechanisms of cellular oxidative signaling are utilised for sustaining life. Altogether, physiological research enables our identification of distinct and precise points of transition from health to the development of multimorbidity throughout the inevitable aging disorders (e.g., diabetes, hypertension, chronic kidney disease, heart failure, peptic ulcer, inflammatory bowel disease, age-related macular degeneration, cancer). With consideration of all organ systems (e.g., brain, heart, lung, gut, skeletal and smooth muscle, liver, pancreas, kidney, eye) and the interactions thereof, this Physiology Series will address the goals of resolving (1) Aging physiology and chronic disease progression (2) Examination of key cellular pathways as they relate to calcium, oxidative stress, and electrical signaling, and (3) how changes in plasma membrane produced by lipid peroxidation products can affect aging physiology, covering new research in the area of cell, human, plant and animal physiology.

Meet the Series Editor



Prof. Dr. Thomas Brzozowski works as a professor of Human Physiology and is currently a Chairman at the Department of Physiology and is V-Dean of the Medical Faculty at Jagiellonian University Medical College, Cracow, Poland. His primary area of interest is physiology and pathophysiology of the gastrointestinal (GI) tract, with a major focus on the mechanism of GI mucosal defense, protection, and ulcer healing. He was a postdoctoral NIH fellow at the University of California and the Gastroenterology VA Medical Center, Irvine, Long Beach, CA, USA, and at the Gastroenterology Clinics Erlangen-Nuremberg and Munster in Germany. He has published 290 original articles in some of the most prestigious scientific journals and seven book chapters on the pathophysiology of the GI tract, gastroprotection, ulcer healing, drug therapy of peptic ulcers, hormonal regulation of the gut, and inflammatory bowel disease.

Meet the Volume Editor



Professor Zaccheaus Awortu Jeremiah is a Professor of Haematology and Blood Transfusion Science at Rivers State University, Port Harcourt, Nigeria. He holds a Doctor of Philosophy (Ph.D.) and other postgraduate degrees from Rivers State University, Port Harcourt. He is a Fellow of three prestigious bodies: the Royal College of Pathologists (FRCPath), London, the Institute of Biomedical Science (FIBMS), London, and the West African Postgraduate College of Medical Laboratory Science (FWAPCMLS). He served as the foundation chair of the Haematology Faculty at the West African Postgraduate College of Medical Laboratory Science (2020-2024). He is the founding president of the Haematology and Blood Transfusion Scientists Society of Nigeria (HBTSSN). He has over 150 publications to his credit.

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Preface

Iron occupies a central position in human biology. It serves as a critical cofactor in fundamental processes such as oxygen transport, cellular respiration, and DNA synthesis. On the other hand, its dual nature of being essential for life and also potentially toxic when misregulated, renders the study of iron metabolism uniquely complex. The maintenance of iron homeostasis requires a finely tuned interplay between absorption, transport, storage, and utilization, and disturbances in this equilibrium underlie a broad spectrum of clinical disorders.

This book, *Human Iron Metabolism - Biology, Health and Disease*, seeks to provide a rigorous and comprehensive account of the biological principles governing iron metabolism and their implications for human health. It integrates molecular and cellular mechanisms with systemic physiology and extends these foundations to the pathophysiology of iron-related diseases. The text is structured to guide the reader from fundamental concepts to translational applications, encompassing:

- Introductory Chapter: Fundamentals of Human Iron Metabolism – An Overview
- Ferritin: Master Regulator of Iron Metabolism in Health and Disease
- Normal Aspects in Blood Transfusion: From Donor to Patient
- Iron Deficiency and Hypothyroidism
- Iron Metabolism and Hemochromatosis: Pathogenic Pathways to Multiple Organ Dysfunction – Clinical and Therapeutic Implications
- Managing Iron Deficiency Anemia in Advanced Chronic Kidney Disease and Dialysis
- Intrauterine Transfusion in the Treatment of Fetal Anemia

The aim of this work is not only to consolidate established knowledge but also to highlight areas of ongoing investigation and debate. Iron metabolism intersects with diverse disciplines, such as nutrition, genetics, immunology, hematology, and neurology, underscoring its relevance across both basic science and clinical medicine. By presenting a synthesis of current understanding alongside unresolved questions, this book aspires to serve as a reference for researchers, clinicians, and students, and to stimulate further inquiry into the multifaceted biology of iron.

In offering this volume, the editor acknowledges the enduring importance of iron as a determinant of human health and disease. I hope that the work will advance scholarship, inform clinical practice, and foster innovation in the study of iron metabolism.

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Chapter 1

Introductory Chapter: Fundamentals of Human Iron Metabolism – An Overview

Zaccheaus Awortu Jeremiah

1. Introduction

Iron is an essential element, vital for numerous physiological processes in the human body. It plays a pivotal role in oxygen transport, energy production, and DNA synthesis. However, maintaining iron homeostasis is crucial, as both iron deficiency and overload can lead to significant health issues [1–3]. This introductory chapter provides an overview of human iron metabolism, highlighting its importance, regulatory mechanisms, and associated disorders.

Iron is integral to several key functions:

a) **Oxygen transport:** Iron is a central component of hemoglobin in red blood cells and myoglobin in muscle cells, facilitating oxygen binding and transport throughout the body.

Iron in hemoglobin transports oxygen through a reversible binding process in red blood cells. Here's how it works, step by step:

Structure of hemoglobin: Hemoglobin is a protein with four subunits, each containing a heme group. At the center of each heme is an iron (Fe^{2+}) atom in the ferrous state.

Oxygen binding (lungs): In the lungs, where oxygen (O_2) partial pressure is high (~100 mmHg), O_2 molecules bind directly to the Fe^{2+} atom in each heme group. This forms oxyhemoglobin: Each Fe^{2+} can bind one O_2 molecule. Fully saturated hemoglobin carries 4 O_2 molecules (one per subunit).

The binding is cooperative: The first O_2 binding changes hemoglobin's shape (from tense "T" to relaxed "R" state), making it easier for the next three O_2 molecules to bind.

Transport in blood: Oxyhemoglobin circulates in arteries. The iron remains in the Fe^{2+} state – it does not oxidize to Fe^{3+} (which would form methemoglobin, unable to bind O_2).

Oxygen release (tissues): In tissues, where O_2 partial pressure is low (~20–40 mmHg), O_2 dissociates from Fe^{2+} due to:

Bohr effect: Lower pH (from $CO_2 \rightarrow$ carbonic acid) and higher 2,3-BPG reduce hemoglobin's O_2 affinity, promoting release. This shifts hemoglobin back to the T state, releasing O_2 for cellular use.

Cycle repeats: Deoxygenated hemoglobin returns to the lungs, binds new O_2, and the process continues.

ETC complex	Iron-containing component	Function
Complex I	Fe–S clusters	Transfers electrons from NADH → ubiquinone
Complex II	Fe–S clusters + heme	Transfers electrons from $FADH_2$ → ubiquinone
Complex III	Heme (cytochrome bc_1)	Electron transfer via Q-cycle
Complex IV	Heme a, Cu centers	Final electron acceptor → reduces O_2 to H_2O

Table 1.
Role in the electron transport chain (mitochondria).

b) **Energy production**: Iron is a constituent of iron-sulfur clusters and heme groups within the electron transport chain, essential for ATP synthesis during cellular respiration. Energy is not directly produced during iron metabolism itself, but iron plays a critical role in energy production within cells, particularly through its involvement in the electron transport chain (ETC) during cellular respiration. Iron metabolism ensures that iron is available in the right form (Fe^{2+}/Fe^{3+}) and location (e.g., mitochondria) to support this process. A summary of this process is shown in **Table 1**.

c) **DNA synthesis:** Iron is involved in the synthesis of DNA, supporting cell division and growth [4–6]. Iron is indispensable in DNA synthesis because it acts as a cofactor for several enzymes involved in nucleotide production and DNA replication:

Ribonucleotide reductase (RNR): This enzyme converts ribonucleotides into deoxyribonucleotides, the building blocks of DNA. RNR contains a diiron center that is essential for its catalytic activity. Without iron, this conversion halts, stalling DNA synthesis.

DNA polymerases: Recent studies show that the catalytic subunits of DNA polymerases contain conserved cysteine-rich motifs that bind iron–sulfur (Fe/S) clusters. These clusters are crucial for the structural integrity and enzymatic activity of the polymerase complex.

DNA repair enzymes: Iron is also vital for enzymes like helicases, nucleases, glycosylases, and demethylases. These enzymes often contain Fe/S clusters that help maintain their structure and function during DNA repair processes.

Iron enables energy production in the following ways:

2. Iron in heme and iron–sulfur clusters

- Iron is a key component of:
  - Heme proteins (e.g., cytochromes in the ETC)
  - Iron–sulfur (Fe–S) clusters (e.g., in complexes I, II, III of the ETC).
- These structures accept and donate electrons, enabling ATP synthesis.

Electrons flow through these iron-containing complexes, creating a proton gradient across the inner mitochondrial membrane.

3. ATP synthesis via oxidative phosphorylation

- The proton gradient drives ATP synthase (Complex V).
- For every glucose molecule:
  - ~30–32 ATP are produced (mostly via ETC).
  - Iron is essential for ~90% of this ATP yield.

Iron must be absorbed, transported, stored, and delivered to mitochondria, asshown in **Table 2**.

In summary: Energy production depends on iron metabolism.

- No ATP is produced directly from iron metabolism directly.
- Iron enables >90% of ATP production via oxidative phosphorylation.
- Iron metabolism supplies functional iron (in heme and Fe–S clusters) to the ETC.
- Disruptions (e.g., iron deficiency anemia) → reduced oxygen delivery + impaired ETC → fatigue, low energy.

Iron metabolism doesn't make energy – it unlocks energy production by fueling the mitochondrial machinery.

Iron never changes valence during normal O_2 transport – it acts as a coordination site, holding O_2 via a loose bond until needed. This prevents oxidative damage while enabling efficient delivery (~1.34 mL O_2 per gram of hemoglobin at full saturation).

Iron absorption primarily occurs in the duodenum, where dietary iron is taken up by enterocytes. There are two forms of dietary iron: a) Heme Iron: Found

Step	Process	Energy link
Absorption	Duodenal enterocytes reduce Fe^{3+} to Fe^{2+} via Dcytb and transport DMT1	Prepares iron for use
Transport	Fe^{2+} is oxidized to Fe^{3+} by hephaestin/ceruloplasmin; it binds to transferrin.	Delivers iron systemically
Cellular uptake	Transferrin receptor → endocytosis → Fe^{2+} released in endosome	Supplies iron to cytosol
Mitochondrial import	Fe^{2+} imported via mitoferrin	Used to synthesize heme and Fe–S clusters.
Heme synthesis	Final step in mitochondria: ferrochelatase inserts Fe^{2+} into protoporphyrin IX → heme.	Heme → cytochromes
Fe–S cluster assembly	ISC (iron–sulfur cluster) machinery in mitochondria	Fe–S → Complexes I–III

Table 2.
Iron metabolism support processes.

in animal products, heme iron is more readily absorbed, with approximately 15% to 35% efficiency. b) Non-Heme Iron: Present in plant-based foods, non-heme iron has a lower absorption rate, around 5%. Once absorbed, iron is transported in the bloodstream bound to transferrin, a glycoprotein that delivers iron to various tissues, including the liver, bone marrow, and spleen. Excess iron is stored in the liver, spleen, and bone marrow in the form of ferritin and hemosiderin. Ferritin serves as the primary storage form, allowing for controlled release of iron when needed. Hemosiderin, an insoluble form of iron storage, accumulates under conditions of iron overload [7, 8].

3.1 Iron balance is tightly regulated through several mechanisms

a) Hepcidin: A key regulator of iron homeostasis, hepcidin is a peptide hormone produced by the liver. It controls iron absorption by binding to ferroportin, the iron exporter on enterocytes, leading to its degradation and reduced iron efflux. Elevated hepcidin levels decrease iron absorption, while low levels increase it (4, 5, 14).

Hepcidin is the master regulator of systemic iron homeostasis, produced primarily by hepatocytes (liver cells). It maintains iron balance by controlling iron release from cells into the bloodstream. Hepcidin inhibits iron export from cells by binding to and degrading ferroportin (the only known cellular iron exporter). The key cells affected by hepcidin are shown in **Tables 3–5**.

Cell type	Role in iron handling	Hepcidin effect
Duodenal enterocytes	Absorb dietary iron	↓ Ferroportin → ↓ iron absorption
Macrophages (spleen, liver)	Recycle iron from old RBCs	Ferroportin → Iron trapped in macrophages
Hepatocytes	Store iron as ferritin	↓ Ferroportin → ↓ **iron release from stores**

Table 3.
Cell types and their roles in handling the hepcidin effect.

Stimulus	Effect on hepcidin	Outcome
↑ Plasma iron (high transferrin saturation)	↑ Hepcidin	↓ Iron release prevents overload
↑ Inflammation (IL-6 → STAT3 pathway)	↑ Hepcidin	↓ Iron in blood limits iron for pathogens (*nutritional immunity*)
↓ Body iron/anemia	↓ Hepcidin	↑ Ferroportin → ↑ iron absorption and release
Erythropoietic demand (e.g., bleeding, hemolysis)	↓ Hepcidin (via erythroferrone from erythroblasts)	↑ Iron supply for hemoglobin synthesis

Table 4.
Hepcidin expression and how it is regulated.

Condition	Hepcidin level	Iron effect
Hereditary hemochromatosis	↓ ↓ Hepcidin (or ineffective)	↑ **Iron absorption → iron overload**
Anemia of chronic disease (ACD)	↑ ↑ Hepcidin (inflammation-driven)	↓ **Iron release → functional iron deficiency**
Iron-refractory iron deficiency anemia (IRIDA)	↑ ↑ Hepcidin (TMPRSS6 mutation)	↓ **Iron absorption → severe anemia**

Table 5.
The clinical relevance of hepcidin.

b) ***Iron-responsive elements (IREs):*** These are sequences in the untranslated regions of mRNA that bind to iron-regulatory proteins (IRPs). Under low iron conditions, IRPs bind to IREs, stabilizing mRNA for iron importers, such as transferrin receptor 1 (TFR1), and inhibiting mRNA for iron storage proteins, like ferritin, thereby enhancing iron uptake and limiting storage [8, 9].

In summary: Hepcidin's role in iron balance

- High hepcidin → locks iron inside cells → ↓ serum iron
- Low hepcidin → releases iron into the blood → ↑ serum iron
- Acts as a negative feedback hormone to prevent both deficiency and overload.

The bottom line is that: Hepcidin is the thermostat of iron metabolism – it senses iron levels, inflammation, and red cell production, then adjusts iron entry and recycling via ferroportin control.

3.2 Imbalances in iron metabolism can lead to various disorders

a) ***Iron deficiency anemia:*** Characterized by insufficient iron to meet physiological needs, leading to reduced hemoglobin production and impaired oxygen transport [10–14].

b) Hereditary Hemochromatosis: A genetic disorder causing excessive iron absorption, leading to iron accumulation in organs such as the liver, heart, and pancreas, potentially resulting in organ damage [5, 6].

Iron deficiency anemia (IDA) represents the end-stage clinical manifestation of chronic negative iron balance, where iron demand exceeds supply over time. This imbalance disrupts iron metabolism at every level – absorption, transport, storage, recycling, and utilization – ultimately impairing hemoglobin synthesis and oxygen delivery. The normal iron balance and the pathophysiology of imbalance are summarized in **Tables 6–10**. The diagnostic confirmation of imbalance and restoration of iron balance are summarized in **Tables 11** and **12**.

Process	Daily flux (mg)	Key players
Absorption	1–2	Duodenal enterocytes, DMT1, ferroportin
Loss	1–2	Skin, gut, menstruation, urine
Recycling	~25	Macrophages → ferroportin → transferrin
Storage	300–1,000	Ferritin (hepatocytes, macrophages)
Utilization	~25	Bone marrow (erythropoiesis)
Steady state: Input = Output + Utilization		

Table 6.
Normal iron balance (homeostasis).

Category	Mechanism	Example
↓ Intake	Low dietary iron (non-heme, plant-based)	Vegetarianism, malnutrition
↓ Absorption	↑ Hepcidin, gut pathology	Celiac, atrophic gastritis
↑ Loss	Chronic bleeding	Menorrhagia, hookworm, GI ulcers
↑ Demand	Growth, pregnancy, erythropoietin therapy	Adolescence, lactation

Table 7.
Causes of negative iron balance.

Stage	Lab finding	Clinical effect
1. Storage depletion	↓ Ferritin (<30 ng/mL)	None (pre-anemic)
2. Iron-deficient erythropoiesis	↓ Serum iron, ↑ TIBC, ↓ transferrin saturation (<16%)	Mild fatigue
3. Iron deficiency anemia	↓ Hb, microcytic hypochromic RBCs	Pallor, fatigue, pica, koilonychia

Table 8.
Sequential stages of iron depletion.

Component	Normal role	In IDA
Hepcidin	↓ during iron need	Inappropriately low or normal, despite low iron (except in cases of inflammation)
Ferroportin	Exports Fe^{2+}	Upregulated in enterocytes/macrophages → max iron release
Transferrin Receptor 1 (TfR1)	Uptake of transferrin-iron	↑ ↑ on erythroblasts → compensatory iron scavenging
Ferritin	Stores Fe^{3+}	↓ ↓ → depleted reserves
DMT1	Apical iron uptake	Upregulated in duodenum

Table 9.
Molecular dysregulation of iron.

System	Effect
Energy Metabolism	↓ Heme and Fe–S clusters → impaired ETC → fatigue
Cognitive	↓ Dopamine synthesis (iron-dependent tyrosine hydroxylase) → irritability, poor focus
Immune	↓ Myeloperoxidase, DNA synthesis → infection risk
Hair/Skin	↓ Cytochrome c → brittle nails, hair loss

Table 10.
Consequences beyond anemia.

Test	Indicates
Serum ferritin <15 ng/mL	Depleted stores
Transferrin saturation <10%	Poor iron supply
↑ sTfR/ferritin ratio	Tissue iron deficit
Reticulocyte Hb <28 pg	Functional deficiency

Table 11.
Diagnostic confirmation of imbalance.

Strategy	Target
Oral iron (ferrous sulfate)	↑ Absorption (with vitamin C)
IV iron (ferric carboxymaltose)	Bypass gut, rapid repletion
Treat underlying cause	Stop blood loss, heal mucosa
Monitor response	Hb ↑ 1–2 g/dL in 3–4 weeks

Table 12.
Restoring iron balance.

4. Pathophysiology of imbalance in IDA

Feedback failure: Low iron → ↓ hepcidin → ↑ absorption/recycling, but it **cannot** compensate for ongoing loss or malabsorption.

In summary: IDA as metabolic imbalance

IDA = Chronic input < output + demand → depleted stores → low serum iron → failed hemoglobin synthesis → microcytic anemia + systemic iron starvation

It is not just low hemoglobin – it is a whole-body iron trafficking failure, where hepcidin fails to adapt, ferroportin overworks, and erythropoiesis starves despite maximal compensatory mechanisms.

4.1 Hereditary hemochromatosis as an imbalance of iron metabolism

Hereditary hemochromatosis (HH) is the archetype of positive iron balance, where uncontrolled iron absorption overwhelms storage and detoxification capacity, leading to progressive tissue iron overload. This imbalance arises from defective

hepcidin-ferroportin regulation, transforming the gut and macrophages into unregulated iron exporters, as shown in **Tables 13–15**.

Core defect: Inappropriately low hepcidin, despite high iron stores → ferroportin hyperfunction.

Feedback failure: Iron sensors (HFE/TfR2) fail to signal the liver → hepcidin stays low → iron flood.

The sequential stages of iron overload, consequences,, and diagnostic hallmarks are summarized in **Tables 16–19**.

In summary: HH as Metabolic Imbalance

HH = Chronic input ≫ output + utilization → Hepcidin deficiency → ferroportin overdrive → transferrin supersaturation → Non-transferrin-bound iron (NTBI) → organ toxicity

It is not just high ferritin – it is a systemic failure of iron restraint, where the liver loses control of the gut-macrophage iron pipeline, flooding tissues with redox-active iron. Early phlebotomy is the only way to reset the balance.

Process	Daily flux	Regulation
Absorption	1–2 mg	Hepcidin ↓ → ferroportin ↑
Recycling	~25 mg	Macrophage ferroportin
Loss	1–2 mg	Obligatory (skin, gut, menses)
Net	~0 mg	Hepcidin = iron thermostat

Table 13.
Normal iron balance.

Gene	Protein	Effect on hepcidin
HFE (C282Y homozygote, ~85% of cases)	MHC-like protein	↓ Hepcidin expression
HJV (Hemojuvelin)	BMP co-receptor	↓ ↓ Hepcidin
TFR2	Transferrin receptor 2	↓ Hepcidin sensing
SLC四十A1	Hepcidin itself	Absent hepcidin

Table 14.
Pathophysiology of imbalance in HHA: Genetic defects (HFE, others).

Component	Normal	In HH
Hepcidin	↑ with iron load	↓ ↓ or absent
Ferroportin	Degraded by hepcidin	Persistent on membrane
Duodenal DMT1/Ferroportin	Tightly controlled	↑ ↑ iron uptake & export
Transferrin saturation	20–45%	>70–90%
Ferritin	30–300 ng/mL	>1000–10,000 ng/mL

Table 15.
Molecular dysregulation.

Stage	Iron deposition	Clinical effect
1. Preclinical	Liver ferritin ↑	None
2. Biochemical	↑ Ferritin, ↑ TSAT	Fatigue, arthralgia
3. Organ Damage	Liver, heart, pancreas, pituitary	Cirrhosis, cardiomyopathy, diabetes, hypogonadism

Table 16.
Sequential stages of iron overload.

Organ	Iron toxicity mechanism	Outcome
Liver	Lipid peroxidation, stellate cell activation	Fibrosis → cirrhosis → HCC risk
Heart	Fe^{2+} → ROS via Fenton reaction	Dilated cardiomyopathy
Pancreas	β-cell damage	Bronze diabetes
Joints	Calcium pyrophosphate deposition	Arthropathy (MCP 2/3)
Pituitary	Gonadotroph damage	Hypogonadism

Table 17.
Tissue-specific consequences.

Test	Finding in HH
Transferrin saturation	>45% (men), >40% (women)
Serum ferritin	>300 μg/L (men), >200 μg/L (women)
HFE genotyping	C282Y homozygote or compound heterozygote
Liver MRI (T2*)	Iron quantification (>125 μmol/g)
Liver biopsy	Pericellular siderosis (Perls' stain)

Table 18.
Diagnostic hallmarks of iron imbalance.

Therapy	Mechanism
Phlebotomy (500 mL weekly)	Removes ~250 mg iron/RBC mass
Target ferritin	<50 μg/L, TSAT <50%
Iron chelation (deferasirox)	For cardiac overload or phlebotomy intolerance
Avoid	Vitamin C (↑ ROS), alcohol, raw shellfish (Vibrio)

Table 19.
Restoring iron balance in HH.

5. Conclusion

Iron metabolism is a complex and finely tuned system essential for human health. Understanding its mechanisms is crucial for diagnosing and effectively treating iron-related disorders. This book has been tailored to meet this objective. Eminent

scholars have contributed the chapters that make up the book. As the editor of the book, I sincerely believe that it will be a valuable resource for physicians, scientists, lecturers, and students.

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Chapter 2

Ferritin: Master Regulator of Iron Metabolism in Health and Disease

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Abstract

Ferritin is a crucial protein for iron storage, recognized for its significant role in maintaining iron homeostasis in humans. Apart from its function in iron sequestration and protection of cells and tissues from oxidative damage, researchers have reported its involvement in some physiological and pathological processes. Structurally, ferritin is a nanocage that contains the heavy and light chains (genes) whose expressions are regulated at multiple levels ranging from transcription to translation to post-translational and epigenetic modifications. These processes are influenced by the body's iron status, cytokines, hypoxia, and other metabolic factors. Our findings include the identification of ferritinophagy, a selective autophagy pathway mediated by nuclear receptor coactivator 4 (NCOA4), as the primary *in vivo* mechanism of iron release. In addition, extracellular ferritin was shown to exert an immunomodulatory effect through receptor-mediated pathways. Advances in single-cell technologies have revealed the structural biology of ferritin, which has also helped for a better understanding of ferritin's dynamic regulation, differences in its isoforms, and tissue-specific functions. Ferritin has become relevant as a biomarker, prognostic tool, and therapeutic target in diseases such as cancer, neurodegenerative diseases, and inflammatory disorders. As a nanocage, it has the potential to be used as a nanocarrier in personalized medicine and targeted therapies. These findings have repositioned ferritin as a multifaceted and functional regulator not only of iron metabolism but also of immune response, oxidative stress, and cellular homeostasis.

Keywords: ferritin, health, oxidative stress, pathology, nanocage, iron

1. Introduction

Ferritin, a highly conserved iron-storage protein, plays a central role in maintaining systemic iron homeostasis. It regulates iron availability for essential

physiological cellular processes such as oxygen transport, deoxyribonucleic acid (DNA) synthesis, and immune function, while preventing iron-induced oxidative damage. Dysregulation of ferritin expression is implicated in a spectrum of pathological conditions, including anemia, hemochromatosis, metabolic syndrome, and cancer. Recent studies revealed ferritin's dual role as both a biomarker and a modulator of disease progression, particularly under inflammatory states where serum ferritin levels may rise independent of iron stores [1, 2]. Understanding ferritin's molecular dynamics is pivotal for advancing diagnostic and therapeutic strategies in iron-related disorders [3].

Iron is an essential nutrient for various biological processes, including oxygen transport, DNA synthesis, and enzyme function [4]. Maintaining iron homeostasis is crucial for health, as both iron deficiency and excess of it can lead to disorders [5]. Iron deficiency anemia is a common condition characterized by fatigue, weakness, and impaired cognitive function [6]. Hemochromatosis, on the contrary, is a genetic disorder characterized by excessive iron absorption and accumulation in organs, leading to tissue damage and an increased risk of various diseases [7].

Iron is a vital element for many living organisms, and its importance cannot be overstated. From oxygen transport to DNA synthesis, iron plays a critical role in many biological processes. However, iron homeostasis is a delicate balance, and its disruptions can lead to various disorders. The importance of iron in human health cannot be overstated. Iron deficiency is a common nutritional disorder that affects millions of people worldwide. However, excessive iron intake can lead to iron overload, which can cause damage to organs such as the liver, heart, and pancreas.

1.1 Overview of iron homeostasis

Iron homeostasis is maintained through a complex interplay of absorption, storage, and utilization. The body regulates iron levels through the hormone known as hepcidin. Hepcidin controls both iron absorption and its release from stores. Hepcidin binds to the iron exporter ferroportin (FPN), causing its internalization and degradation, thereby reducing iron absorption and increasing iron storage. When iron levels are low, hepcidin levels decrease, allowing for increased iron absorption and release from stores [8].

The regulation of iron homeostasis is a complex process involving multiple proteins and pathways. Hepcidin is a key player in this process, and its dysregulation can lead to various iron-related disorders. Understanding the mechanisms of hepcidin regulation is essential for developing effective treatments for iron-related disorders.

1.2 Significance of iron in biological systems

Iron plays a critical role in various biological processes as described below.

Iron is a key component of hemoglobin and myoglobin, which are proteins that transport oxygen in the blood and biological muscles, respectively. Iron is required for the activity of enzymes involved in DNA synthesis and repair, such as ribonucleotide reductase [9]. Iron is a cofactor for various enzymes, including cytochromes, which play a crucial role in energy production and detoxification [10]. The importance of iron in biological systems cannot be overstated. From oxygen

transport to DNA synthesis, iron plays a vital role in many biological processes. Understanding the role of iron in these processes is essential for developing effective treatments for iron-related disorders.

1.3 Challenges of iron toxicity and deficiency

Iron toxicity can lead to oxidative stress and tissue damage, while iron deficiency can cause anemia, impaired cognitive function, and other disorders [11]. Iron toxicity can occur due to genetic disorders such as hemochromatosis or due to excessive iron intake. Iron deficiency, on the contrary, can result from inadequate dietary intake, increased requirements, or chronic blood loss [4]. Both iron toxicity and deficiency can have serious consequences for human health. Understanding the causes and consequences of these disorders is essential for developing effective treatments. Treatment options for iron-related disorders depend on the underlying cause and severity of the condition.

1.4 Ferritin as a central player

Ferritin is a protein complex that plays a crucial role in iron storage and detoxification. It stores excess iron in a safe and bioavailable form, preventing oxidative stress and tissue damage [12]. Ferritin is composed of 24 subunits that form a hollow shell, which can store up to 4,500 iron atoms [13]. Ferritin levels are often used as a marker of iron stores in the body [14]. The role of ferritin in iron storage and detoxification is essential for maintaining iron homeostasis. Its dysregulation can lead to various iron-related disorders. Understanding the mechanisms of ferritin regulation is essential for developing effective treatments for iron-related disorders.

1.5 Historical discovery and evolution of understanding

Ferritin was first discovered in the 1930s by Laufberger and colleagues, who isolated and crystallized the protein from horse spleen [15]. Since then, research has elucidated the structure, function, and regulation of ferritin, revealing its importance in iron metabolism. Studies have shown that ferritin plays a critical role in maintaining iron homeostasis and protecting against iron toxicity. The discovery of ferritin has led to a greater understanding of iron metabolism and its role in human health. Further research is needed to fully elucidate the mechanisms of ferritin's action and its potential therapeutic applications.

1.6 Dual role in iron storage and detoxification

Ferritin's dual role is essential for maintaining iron homeostasis as described below.

Ferritin stores excess iron in a safe and bioavailable form, allowing for its release when needed. Ferritin sequesters excess iron, preventing oxidative stress and tissue damage. By storing excess iron, ferritin helps to maintain iron homeostasis and protect against iron toxicity [16]. Ferritin and iron homeostasis are closely linked, and understanding their relationship is essential for developing effective treatments for iron-related disorders. In conclusion, iron is an essential nutrient that plays a critical role in various biological processes. Maintaining iron homeostasis is crucial for health, and disruptions can lead to various disorders. Ferritin is a key player in

iron storage and detoxification, and its dysregulation can lead to iron-related disorders. Understanding the mechanisms of iron homeostasis and ferritin regulation is essential for developing effective treatments for these disorders.

2. Structural overview of ferritin

Ferritin, as an iron-storage protein, is found in prokaryotic and eukaryotic organisms. In humans, it forms a hollow spherical shell composed of 24 protein subunits, folding into an outer diameter of 12–13 nm and an internal diameter of 8 nm capable of storing up to 4,500 iron atoms as ferrihydrite-like minerals [17]. This nanocage architecture is exceptionally efficient because it minimizes free, reactive iron while providing a reservoir for metabolic needs [18]. In the following subsections, we will discuss in detail the structure of ferritin.

2.1 Subunit composition and biochemistry

Ferritin consists of two major subunits in the human body, referred to as isoferritins, which are the heavy chain (H; 21 kD) and light chain ferritin (L; 19 kD). The heavy chain ferritin is present in the kidney, brain, and heart, while the light chain ferritin can be seen in the liver, spleen, and bone marrow. Heavy chain ferritin possesses ferroxidase activity, catalyzing ferrous (Fe^{2+}) to ferric (Fe^{3+}). This conversion is crucial for iron detoxification via the Fenton reaction. Light chain ferritin lacks this catalytic activity but accelerates iron nucleation, storage-centric needs, and mineral core formation. These two subunits are present on chromosomes 11 and 19 and have different promoter regions. As much as they are different subunits, they have a similar sequence identity up to 55%, a similar four-helix bundle fold plus a fifth helix, yet differ in active-site architecture, with the H having two metal-coordinating residues, while L replaces them with salt-bridge residues [19].

2.2 Assembly and pore architecture

Ferritin is a fairly large molecule that assembles into an icositetramer (24-mer). It safeguards the iron mineral inside its hollow nanocage, guarding it from the cytosol and only releasing it when there is a cellular need. It connects to the surrounding environment through its symmetric pores and channels and has threefold and fourfold symmetry [20]. Threefold pores or channels (42–58Å, hydrophilic) are eight in number, located at the corners and they serve as a channel for iron entry and exit whereas the fourfold channels (40–60Å, hydrophobic) are six in number, located at the faces of the iron cube, they remain gated, possibly regulating water or small ligand passage [21, 22]. The threefold channel is made up of hydrophilic residues of histidine, aspartate, and glutamic acid, while the fourfold channel is made up of 16 residues of leucine and glutamine. Additionally, the ferroxidase center resides in the H-subunit at dimer interfaces, binding di-iron substrates. Iron enters through threefold channels, is oxidized in H-centers, migrates to L-rich areas for mineral nucleation, and accumulates as a crystalline core. A simple schematic diagram is provided to describe ferritin shell assembly (see **Figure 1**).

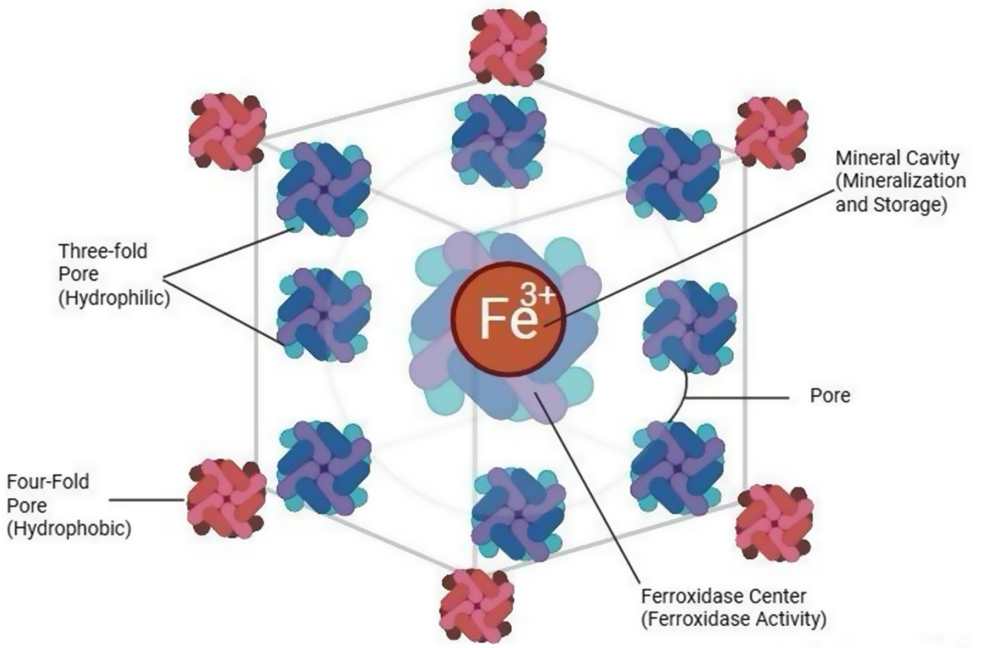


Figure 1.
Ferritin shell, highlighting three/fourfold pores, ferroxidase center, and mineral cavity. This diagram illustrates the structural organization and functional features of the ferritin nanocage. Iron enters through hydrophilic threefold pores and hydrophobic fourfold pores, where it undergoes oxidation at the ferroxidase center ($Fe^{2+} \rightarrow Fe^{3+}$). The oxidized iron ($Fe^{3+}$) is subsequently stored within the mineral cavity in a safe, bioavailable form until required for cellular processes.

2.3 Genetics and subcellular distribution

Ferritin, as an important protein in iron metabolism, is present in several cellular compartments of the body, which include mitochondria, nucleus, and cytosol and are considered the most ferritin-abundant compartment. Cytosolic ferritin is present in all tissue cells, making ferritin more structurally and biochemically necessary for cell vitality. Its functional genes are the ferritin heavy chain (FTH1) gene present on chromosome 11 and the ferritin light chain (FTL) gene present on chromosome 19, coding for the heavy and light chains, respectively. The cytosol is the primary location for iron buffering. Nuclear ferritin is seen in the hollow spherical structure like a compact nucleus of iron separated from the cytoplasm, where the H-rich variant translocates into the nucleus in response to stress or metabolic signal, protecting the DNA from oxidative damage. In the nucleus, iron remains soluble, and it is only released when it is needed for biological activities. The last is the mitochondrial ferritin (FTMT), which is encoded by the intron-free gene FTMT located on chromosome 5. This precursor is composed of 242 amino acids; the N-terminal driving sequence is cleaved, and after the cleavage of the peptide, the subunits are assembled into a subunit homopolymer molecule. This helps in safeguarding mitochondrial iron homeostasis. Despite the similarities of the cytosolic ferritin to FTMT in function, such as iron storage and detoxification, the expression

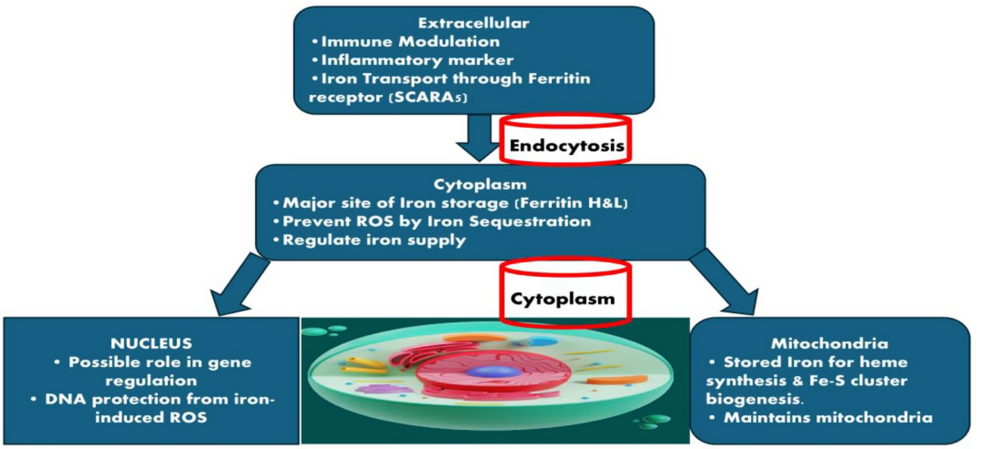


Figure 2.
Cellular localization and multifunctional roles of ferritin. Ferritin exerts functions across different compartments: in the extracellular space, it participates in immune modulation, acts as an inflammatory marker, and mediates iron transport via scavenger receptor class A member 5 (SCARA5) receptors. Through endocytosis, ferritin enters the cytoplasm, where ferritin H- and L-chains act as the major iron storage system, sequestering iron to prevent reactive oxygen species (ROS) generation and regulating its supply. In the nucleus, ferritin may contribute to gene regulation and protect DNA from iron-induced oxidative stress. In mitochondria, FTMT stores iron for heme synthesis and Fe–S cluster biogenesis, ensuring mitochondrial integrity and function.
In summary, in cytosol (rich in H–L heteropolymer, iron storage and detoxification), mitochondria (rich in H homopolymer, safeguarding cellular powerhouses), and nucleus (predominantly H-rich, DNA defense and epigenetic modulation), ferritin has critical functions in maintaining cellular iron balance and mitigating oxidative stress.

of FTMT is restricted to particular cell types [23]. The role of ferritin in different cellular components is illustrated in **Figure 2**.

2.4 Functional consequences of structure

These dimers or subunits form the building blocks in the ferritin self-assembly process and are also of significant physiological function, displaying tissue-specific adaptive process that imparts distinctive tissue-specific functions. The interplay of H- and L-subunits underlies functions in ferroxidase catalysis, where the H-subunit's di-iron center oxidizes Fe^{2+} using oxygen (O_2) or hydrogen peroxide (H_2O_2), yielding Fe^{3+} for storage. There is also core formation and iron storage, which is a specific function of the L-subunit through facilitating mineral growth, optimizing core density, and enabling efficient iron sequestration. Moreover, in detoxification by oxidizing iron within the safe confines of the shell and preventing free iron accumulation, ferritin minimizes ROS creation via the Fenton reaction. Finally, adaptive variation is executed by different H:L ratios; they tailor iron processing to organ-specific needs, including fast processing in brain/muscle and ample storage in liver/spleen [24].

2.5 Emerging insights and research opportunities

Ferritin's nanocage is being exploited as a nanocarrier for drug delivery or imaging agents owing to its size, stability, and modifiability. Ferritin has firm

properties that show its ability in self-assembly and has an intricate, tight structure and control mechanism. These properties qualify ferritin as a potential nanocarrier for the delivery of substances around the body. Ferritin cores exhibit superparamagnetism; high-field studies suggest novel electron transport or magnetic properties potentially relevant to biophysics. The role of nuclear ferritin in genome maintenance and repair has great scope for further study. Consequently, studying how regulatory processes influence the self-assembly of large protein complexes may help improve techniques for therapeutic applications.

3. Regulation of ferritin production

The regulation of ferritin production is carried out through a tight control system that controls intracellular iron levels. The proteins responsible are called the iron regulatory proteins. Regulation of ferritin can also be influenced by some other signaling factors like cytokines (tumor necrosis factor, interferon-γ, interleukin-1 alpha, and interleukin-6), and oxidative stress. These processes include but are not limited to transcriptional regulation, translational control, post-translational modification, inflammatory processes stimulated by inflammatory cytokines, and oxidative stress. Ferritin can also be regulated by hypoxia, hormonal and metabolic influences, epigenetic mechanisms, and integrated signaling networks due to the interaction of ferritin metabolic proteins such as divalent metal transport, transferrin, FPN, and hepcidin as shown in **Figure 3**.

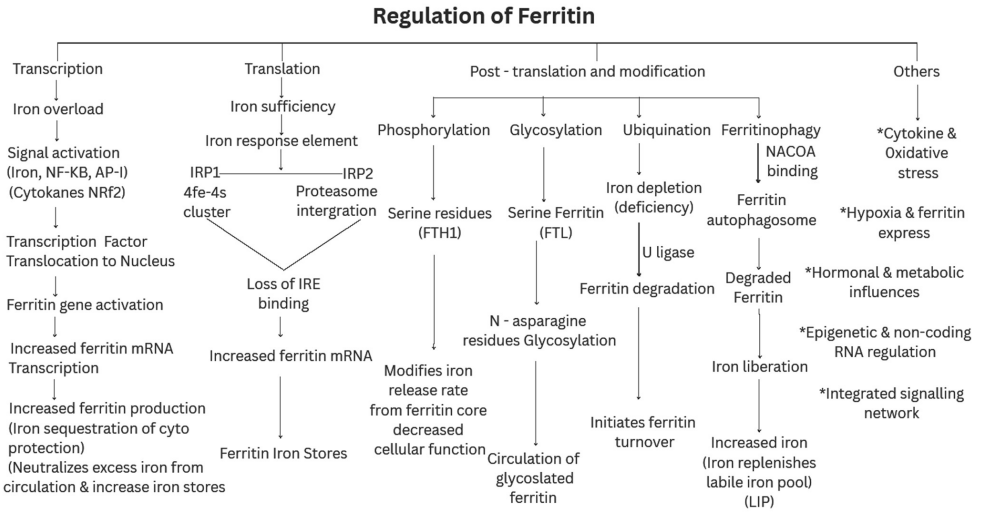


Figure 3.
Showing regulation of circulatory iron and ferritin production. Ferritin regulation occurs at multiple levels: transcription, translation, post-translational/modification, and other influences. During iron overload, signals such as iron itself, NF-κB, AP-1, or cytokines activate transcription factors that move to the nucleus, increasing ferritin mRNA and protein production to store excess iron safely. At the translation level, iron sufficiency triggers iron response elements (IREs) and iron regulatory proteins (IRP1/IRP2), which modulate ferritin synthesis and proteasome degradation to maintain iron balance. Post-translational modifications like phosphorylation, glycosylation, and ubiquination control ferritin stability, turnover, and iron release. Additionally, ferritin can be degraded in autophagosomes (ferritinophagy) to release iron when needed. Other factors, including cytokines, oxidative stress, hypoxia, hormonal and metabolic signals, epigenetic regulation, and integrated signaling networks, further influence ferritin function and regulation.

3.1 Transcriptional regulation of ferritin genes

Transcriptional regulation of ferritin is influenced by cellular iron status and a variety of signaling molecules, including cytokines, hormones, and oxidative stress-related transcription factors. The genes encoding ferritin subunits FTH1, FTL, and FTMT contain regulatory promoter elements responsive to transcription factors such as nuclear factor kappa B (NF-κB), activator protein-1 (AP-1), and nuclear factor erythroid 2 related factor 2 (Nrf2) [25]. Inflammatory or oxidative stress conditions cause Nrf2 to translocate to the nucleus, binding to antioxidant response elements (AREs) in ferritin gene promoters, causing the upregulation of transcription. Interleukin-6 (IL-6) stimulates FTH1 transcription through the signal transducer and activator of transcription 3 (STAT3) pathway, contributing to increased ferritin levels during acute-phase reactions [26, 27]. This transcriptional control allows for rapid production of ferritin when cells are under stress or exposed to elevated iron levels.

3.2 Translational control: The iron-regulatory protein and iron-responsive element system

The primary translational mechanism regulating ferritin synthesis involves the iron-responsive element (IRE) and iron-regulatory protein (IRP) system as shown in **Table 1**. Iron regulatory proteins bind to the ribonucleic acid (RNA) stem loops, which contain the IRE in their untranslated region to block or modulate the translation of the ferritin messenger RNA (mRNA) [28]. As iron becomes available, IRP1 acquires a [4Fe–4S] cluster and loses IRE-binding capacity, while IRP2 undergoes proteasomal degradation. This switch allows for rapid translation of ferritin mRNAs in response to iron sufficiency [29, 30]. This mechanism ensures that ferritin protein levels are modulated not only by gene expression but also by real-time cellular iron status.

3.3 Post-translational modifications

Beyond transcription and translation, ferritin can undergo post-translational modifications such as phosphorylation, glycosylation, and ubiquitination. These modifications can influence ferritin's stability, intracellular localization, and degradation. For instance, phosphorylation of specific serine residues in the heavy chain may affect iron release rates. Glycosylated ferritin, particularly found in serum, may play a distinct role in iron signaling and immune interactions [31]. Additionally, under stress conditions, ubiquitin ligases target ferritin for degradation via ferritinophagy, a selective autophagy pathway mediated by nuclear receptor coactivator 4 (NCOA4), which maintains iron homeostasis by causing ferritin iron storage or release according to demand [32, 33].

3.4 Regulation by inflammatory cytokines and oxidative stress

Ferritin is an acute-phase reactant, and its synthesis is significantly upregulated in response to pro-inflammatory cytokines such as IL-1β, TNF-α, and IL-6. These cytokines enhance ferritin production through STAT3 and NF-κB

Regulatory level	Mechanism	Functional impact	References
Transcriptional control	Activation via transcription factors (e.g., NF-κB, Nrf2, HIF-1α)	Increases ferritin H-chain expression under stress or hypoxia	[41]
	B3 transcription factors modulate ferritin gene expression in development	Tissue-specific iron distribution and ferritin isoform balance	[42]
Cytokines, hormones, oxidative stress	TNF-α, IL-1α, and IFN-γ induce ferritin transcription via NF-κB and JunD pathways	Enhances ferritin synthesis during inflammation and oxidative stress	[43, 44]
Translational control	Iron-responsive elements (IREs) in 5′ -UTR bind IRPs	Suppresses ferritin translation under low iron; derepression under high iron	[45]
	eIF3 binds adjacent to IRE to repress FTL translation	Adds an additional layer of translational control, disrupted in hyperferritinemia	[45]
Feedback mechanisms	IRP1/IRP2 binding to IREs modulated by intracellular iron levels	Maintains iron homeostasis by adjusting ferritin and transferrin receptor synthesis	[30, 46]
	NCOA4-mediated ferritinophagy regulates ferritin turnover.	Releases stored iron during deficiency; prevents overload	[47]
Post-translational modifications	Phosphorylation of ferritin subunits	Alters stability and degradation rate	[48]
	Glycosylation of serum ferritin	Affects secretion and immunoreactivity; linked to inflammation	[49]

Table 1.
Regulation of ferritin expression.
NF-κB: nuclear factor kappa-light-chain-enhancer of activated B cells;) Nrf2: nuclear factor erythroid-2-related factor 2; HIF-1α: hypoxia-inducible factor 1-alpha; TNF-α: tumour necrosis factor-alpha; IL-1α: interleukin-1 alpha; IFN-γ: interferon-gamma; IRE: iron-responsive element; IRP: iron regulatory protein; FTL: ferritin light chain; eIF3: eukaryotic initiation factor 3; NCOA4: nuclear receptor coactivator 4; FtMt: mitochondrial ferritin; UTR: untranslated region.

signaling. Simultaneously, oxidative stress activates Nrf2, which upregulates ferritin transcription [34]. The increase in ferritin serves as a protective mechanism, sequestering iron and limiting its role in catalyzing ROS formation. Thus, ferritin acts both as a marker and mediator of inflammation.

3.5 Hypoxia and ferritin expression

Hypoxia induces ferritin expression through hypoxia-inducible factors (HIFs), particularly HIF-1α, which stabilizes under low oxygen tension and binds to hypoxia-responsive elements (HREs) within the ferritin FTL gene promoter region, which leads to ferritin transcription. This regulation enables cells to adapt to iron-limited environments commonly found in hypoxic tissues, such as tumors or inflamed areas. Ferritin helps reduce ROS formation by storing iron safely, protecting cells during hypoxic stress [35].

3.6 Hormonal and metabolic influences

Hormones like insulin, estrogens, and glucocorticoids can modulate ferritin levels. For example, estrogens increase FTL transcription, while glucocorticoids may enhance both FTH and FTL gene expression under stress. Metabolically, iron-sensing hormones like hepcidin influence systemic iron levels, indirectly affecting ferritin expression. High hepcidin levels promote iron sequestration in tissues, raising intracellular ferritin synthesis to store accumulated iron. Accumulating evidence implicates elevated serum ferritin and disruptions in iron metabolism as potential elements linked to an increased risk of metabolic syndrome [4, 36].

3.7 Epigenetic and non-coding RNA regulation

Recent evidence suggests that micro-RNAs (miRNAs) and long non-coding RNAs (lncRNAs) also contribute to ferritin regulation. For instance, miR-200b targets FTL mRNA, suppressing its translation, while lncRNA PVT1 may modulate ferritin expression indirectly via regulation of IRP/IRE axis components. Epigenetic modifications such as DNA methylation in ferritin gene promoters can alter transcription in cancer and aging [37, 38].

3.8 Integrated signaling networks affecting ferritin expression

Ferritin regulation is embedded within larger iron-responsive signaling networks, including interactions with hepcidin, transferrin receptor (TfR), divalent metal transporter 1 (DMT1), and FPN. These systems coordinate iron uptake, export, and storage [39]. Additionally, pathways like mechanistic target of rapamycin (mTOR) and AMP protein kinase (AMPK) influence ferritin translation through metabolic stress responses. Crosstalk between redox balance, metabolic cues, and immune signals tightly tunes ferritin production [40].

4. Ferritin in iron metabolism

4.1 Iron storage and release

Iron is vital for a wide array of cellular functions, including oxygen transport, DNA synthesis, and mitochondrial energy production. However, because iron is redox-active and can catalyze the formation of ROS via Fenton chemistry, its storage must be tightly regulated. Ferritin, a ubiquitous and highly conserved protein, is the primary intracellular iron storage molecule. It protects cells by sequestering excess iron in a non-reactive ferric (Fe^{3+}) form within its hollow, proteinaceous nanocage [50, 51].

The process of iron sequestration begins when Fe^{2+} enters ferritin through hydrophilic channels located at its threefold symmetry pores. Once inside, Fe^{2+} is oxidized at the ferroxidase center to Fe^{3+} and directed toward the central cavity, where it is incorporated into the mineral core. This conversion is vital because ferrous iron, if left unregulated, can participate in Fenton reactions,

generating hydroxyl radicals (•OH), which damage DNA, proteins, and membranes [52].

Ferritin synthesis is tightly regulated by the IRE/IRP system. In iron-rich environments, IRPs dissociate from IREs in the 5′ -untranslated region (UTR) of ferritin mRNA, allowing translation to proceed and ferritin levels to rise. When iron is scarce, IRPs bind to IREs and inhibit ferritin translation, conserving cellular iron [53].

The stored form of iron ferritin is not merely a passive iron sink; it participates dynamically in iron flux, releasing iron when required to maintain metabolic processes [4, 54]. Under iron-deficient conditions or during periods of high cellular demand such as erythropoiesis, infection, or rapid cell proliferation, ferritin undergoes selective degradation through a process termed ferritinophagy. This autophagy-mediated mechanism is facilitated by the cargo receptor NCOA4, which binds ferritin and delivers it to autophagosomes for lysosomal degradation. The iron released is then recycled back into the labile iron pool (LIP) [55].

Overall, ferritin functions as a buffering system for intracellular iron. It captures and immobilizes free iron during periods of excess, preventing iron-mediated oxidative stress, and releases iron in a controlled fashion when needed to support essential cellular functions. This dual role makes ferritin indispensable in iron metabolism and vital in both health and disease states [3].

4.2 Dynamic equilibrium between ferritin-bound iron and LIP

Iron homeostasis is not only dependent on total iron content but also on the form in which iron exists, whether it is ferrous or ferric iron. The most important factor to consider in the equilibrium is the interplay between ferritin-bound iron, which is inert and stored safely, and the LIP, which is readily available for metabolic use. The LIP consists of low-molecular-weight, redox-active iron, mainly Fe^{2+} complexed with small ligands such as citrate or adenosine triphosphate (ATP). While the LIP is essential for iron-requiring cellular functions, it also poses a risk due to its potential to catalyze harmful Fenton reactions [56, 57].

Ferritin acts as a dynamic buffer, absorbing excess iron from the LIP and releasing it when iron is needed. This bidirectional flow ensures that the LIP remains within a physiologically safe range. When intracellular iron levels rise, ferritin synthesis is upregulated (via IRP/IRE mechanisms), increasing its capacity to sequester iron and lower the LIP. Conversely, under iron-depleted conditions, ferritin is degraded primarily through ferritinophagy to replenish the LIP and sustain vital iron-dependent cellular functions [58].

Iron-sensitive studies carried out by Ma and colleagues [59], using fluorescent probes and metal chelators, have revealed how fluctuations in LIP concentrations are linked to ferritin metabolism. An example is the treatment of cells with iron salts, which rapidly increases LIP levels, followed by a delayed but robust rise in ferritin synthesis and iron sequestration. In contrast to the above, iron chelation reduces LIP levels and promotes ferritin degradation, especially in lysosomes to liberate iron into circulation [60].

This equilibrium is not merely a passive chemical exchange but is actively regulated by multiple cellular signals. Stress responses such as hypoxia, oxidative

stress, and inflammation all influence ferritin expression and degradation, and therefore modulate LIP levels. For instance, during inflammation, cytokines like IL-6 promote ferritin transcription through STAT3 activation, reducing free iron and depriving pathogens of a growth resource, an aspect of nutritional immunity [61].

Disruption of this balance contributes to pathologies. Thus, the dynamic relationship between ferritin and the LIP is fundamental to iron metabolism. Ferritin's ability to modulate iron availability while minimizing toxicity illustrates its sophisticated role as a central player in intracellular iron homeostasis.

4.3 Mechanisms of iron mobilization from ferritin

Although the role of ferritin in iron sequestration is well characterized, its ability to release iron is also very important. Cellular survival and proliferation depend on timely access to iron for essential processes such as DNA synthesis, oxygen transport, and mitochondrial respiration. Thus, ferritin must not only store iron safely but also enable its controlled mobilization in response to physiological needs.

There are two major mechanisms of iron release from ferritin as described below.

4.3.1 Non-enzymatic iron release

In vitro studies have demonstrated that ferritin can release iron when exposed to reducing agents such as flavins, ascorbate, nicotinamide adenine dinucleotide (NADH), and thiol compounds (e.g., glutathione). These reductants convert Fe^{3+} within the ferritin core to the more soluble Fe^{2+}, which can then diffuse out through the protein's hydrophilic channels. While informative, this process is relatively inefficient and likely not the dominant mechanism in living cells due to slow kinetics and lack of regulation [62–65].

4.3.2 *Ferritinophagy: The dominant* in vivo *pathway*

The primary physiological mechanism for iron release from ferritin is a selective form of autophagy known as ferritinophagy. This process is mediated by the cargo receptor NCOA4. In the event of iron-deficient conditions or during increased cellular demand, NCOA4 binds cytosolic ferritin and reduces it to ferritin autophagosomes. The autophagosomes then fuse with lysosomes, where ferritin is degraded by lysosomal enzymes, thereby releasing iron into the cytoplasm. Released iron reenters the LIP, becoming bioavailable for essential metabolic reactions. This process is tightly regulated, wherein later NCOA4 itself is degraded in iron-replete states, limiting ferritin turnover and avoiding unnecessary iron release [33, 66].

Some proteins have been implicated in facilitating iron exit from lysosomes following ferritin degradation, like the lysosomal permeases and proton pumps, which help maintain pH gradients that make iron freely soluble. Other mechanisms that may drive ferritinophagy includes mTOR signaling, hypoxia, and oxidative stress. A clear understanding of ferritinophagy provides insight into diseases of iron dysregulation and presents potential therapeutic targets [67, 68].

4.4 Interaction with other iron proteins

Ferritin works in conjunction with other iron-regulating proteins to maintain systemic and cellular iron homeostasis. These interactions form a highly integrated network that determines iron absorption, storage, utilization, and export. The key players in this network include transferrin, FPN, hepcidin, and divalent metal transporter 1. Ferritin plays both responsive and regulatory roles in this system, contributing to an iron feedback loop that adapts to physiological needs.

4.4.1 Ferritin and transferrin: Iron acquisition and buffering

Transferrin is the main extracellular iron transport protein, delivering iron to tissues via TfR1. Once iron is internalized through receptor-mediated endocytosis, Fe^{3+} is released in the acidic endosome, reduced to Fe^{2+} by six transmembrane epithelial antigen of the prostate (STEAP3), and transported into the cytoplasm by DMT1 [69]. As a buffering protein, ferritin captures excess cytosolic iron, thus buffering transient spikes in iron levels. This interaction is critical because unbuffered iron would otherwise increase the LIP, posing oxidative risk, which may lead to cellular damage. By modulating the LIP, ferritin indirectly regulates the expression of transferrin receptor: when ferritin sequesters more iron, LIP drops, leading to upregulation of TfR1 via the IRP/IRE system, promoting more iron uptake [70]. Therefore, expression of TfR1 and ferritin is inversely regulated via the IRP/IRE system. Low intracellular iron enhances TfR1 and suppresses ferritin synthesis; high iron does the opposite, favoring ferritin accumulation to curb overload.

4.4.2 Ferritin and FPN: Gatekeeping iron export

FPN is an iron exporter in mammalian cells and is present primarily in macrophages, hepatocytes, and enterocytes. While ferritin sequesters iron intracellularly, FPN enables its release into the bloodstream, especially during iron recycling by macrophages or absorption from the intestine [71]. The relationship between ferritin and FPN is both complementary and compensatory. In macrophages, ferritin stores iron recycled from senescent red blood cells (RBCs), while FPN exports this iron back into circulation. Under inflammatory conditions, ferritin expression increases while FPN is downregulated, creating an intracellular iron trap, a mechanism thought to limit iron availability to pathogens during infection [72].

4.4.3 Ferritin and hepcidin: Coordinated regulation of systemic iron

Hepcidin is a 25-amino acid peptide hormone produced in the liver; it serves as the central regulator of systemic iron balance. It binds to FPN, triggering its internalization and degradation, thereby reducing iron export from enterocytes and macrophages [73]. Hepcidin indirectly affects ferritin dynamics. When hepcidin levels are high during inflammation or iron overload, cellular iron increases due to FPN inhibition. This results in ferritin upregulation to accommodate the surplus iron. Conversely, in hepcidin-deficiency disorders (e.g., hereditary

hemochromatosis (HH)), FPN remains active, systemic iron overload ensues, and ferritin levels rise as a defensive response to protect tissues from iron toxicity [74].

Ferritin's (storage) interactions with transferrin (import), FPN (export), hepcidin (regulator), and associated proteins form an intricate network that governs iron metabolism at both the cellular and organismal levels. These relationships enable ferritin to play a responsive and central role in the iron economy, buffering excess iron, releasing it on demand, and adapting to systemic iron signals.

4.5 Role in systemic iron distribution

Iron distribution in the human body is a tightly orchestrated process, ensuring that all tissues receive sufficient iron for their metabolic needs without risking toxicity. Ferritin, though primarily an intracellular protein, plays a critical role in this systemic regulation by influencing the flow of iron between absorption, transport, storage, and utilization. While transferrin and FPN mediate the physical movement of iron, ferritin determines whether iron is stored, mobilized, or restricted within tissues, thereby shaping overall iron distribution across organ systems [39].

Systemic iron distribution involves the following major compartments: the intestine, where iron is absorbed primarily in the duodenum. Ferritin levels in enterocytes reflect iron status and regulate absorption. When iron is abundant, enterocytes increase ferritin expression, trapping iron and preventing excess entry into the bloodstream. Iron retained in ferritin is ultimately lost when enterocytes are shed, a natural mechanism of iron excretion. In the liver, ferritin acts as the primary iron storage protein. Hepatocytes store iron in ferritin or hemosiderin and serve as a buffer against systemic fluctuations. Ferritin in the liver reflects both current intake and long-term iron load. In the macrophages present particularly in the spleen and liver, macrophages recycle iron from aged RBCs. Ferritin stores iron temporarily until it is exported via FPN. Consequently, in the bone marrow, the largest iron consumer, ferritin supports erythropoiesis by maintaining intracellular iron supply during rapid heme synthesis. Finally, in the brain, although protected by the blood–brain barrier, iron still enters the central nervous system (CNS) through tightly regulated mechanisms. Ferritin expression in astrocytes and microglia helps buffer iron levels and prevent neurotoxicity [71, 72, 74].

5. Ferritin in physiological processes

Ferritin is critical to homeostasis as its primary function is iron storage and making iron available for critical cellular processes [75]. It is the intracellular iron storage protein that stores excess iron after all the cellular needs of the body are met and releases it for cellular activities during an acute demand [3]. It also protects lipids, DNA, and proteins from the potentially toxic effects of iron [75]. The ferric form of iron, which is linked to phosphate and hydroxide anions, is kept within the ferritin molecule [76]. The maximum number of iron atoms that each ferritin molecule may sequester is about 4,500. Ferritin also contains enzymatic properties that allow iron to be internalized and stored in the ferritin mineral core, transforming ferric iron to ferrous iron. Ferritin is also found in trace amounts in human serum, and it is higher in inflammatory and iron overload states [77, 78]. An essential part of maintaining iron homeostasis is ferritin. Its main function is iron

sequestration; as a ferroxidase, it transforms iron (II) into iron (III) as iron is absorbed and stored in the ferritin mineral core.

Because iron can produce reactive species that can directly harm proteins and DNA, it is hazardous in cellular systems. Ferritin is essential to an organism's existence because it buffers and captures the intracellular iron pool [75].

5.1 Ferritin in erythropoiesis and heme synthesis

Rapid accumulation of large amounts of iron is required for terminal differentiation of erythropoietic progenitors, and these are transported to the mitochondria where it is incorporated into the heme [79]. According to a study that followed the movement of radiolabeled Fe–Tf through reticulocytes that were separated, iron first accumulated in ferritin before showing up in hemoglobin [80]. Iron was transported to ferritin even when Fe–Tf absorption was limited to extremely low levels, suggesting that ferritin iron constituted an essential component of iron trafficking across the cell rather than the overflow from excessive buildup. The fact that radioiron delivered as Fe–Tf first arrived in ferritin before emerging in hemoglobin was confirmed by a subsequent work employing early erythroid precursors separated from peripheral blood [81]. As erythrocytes grow, ferritin levels appear to degrade and release ferritin iron, as evidenced by their rapid surge early in terminal differentiation and subsequent decline [80].

For the flux of iron through ferritin in developing cells, the poly rC-binding protein 1(PCBP1) and the autophagic cargo receptor for ferritin known as NCOA4 are required as chaperones [79].

The transcriptional and translational levels of IRPs and IREs govern intracellular iron homeostasis [82]. The two subtypes of IRP, IRP1 and IRP2, are both controlled by the amounts of oxygen and iron in cells [82–84]. When iron levels are low, IRP interacts more effectively with the IREs on the 5′-UTR or 3′-UTR of the mRNAs of iron-related genes (e.g., TfR1, DMT1, ferroportin 1 (Fpn1) etc.). Ferritin L and H mRNAs' 5′-UTR IRP-IRE interaction prevents ferritin translation [53]. One of the most significant biological indicators of inflammation, ferritin also serves as a gauge of iron status. Cytokine-mediated increase in ferritin H and L production occurs during inflammation, both at the transcriptional and translational levels [3].

5.2 Ferritin in immune function and modulation of immune responses

Recent research has found that there is a strong correlation between elevated ferritin levels and disease severity and prognosis. Ferritin is an essential component of inflammation, aiding in the generation of cytokines, immunological activation, and the advancement of disease. For cell survival and proliferation during an infection, iron must remain available to both the host and the pathogen [3]. It has been shown that changes in cellular and serum iron are significant indicators of disease [85]. A readily available biomarker, ferritin, has been the subject of much research due to its significance in iron metabolism and its potential as a vital sign of infectious disease. By promoting iron delivery and storage, this protein plays a critical role in maintaining iron homeostasis [86]. Numerous variables, including oxidative stress and inflammatory stimulation, influence its expression [87]. Ferritin might actively support inflammation rather than only being an epiphenomenon of the acute-phase response. The multifunctional protein ferritin, which sits at the intersection of

inflammation and immunity, is produced by the liver in response to pro-inflammatory cytokines after certain tissue damage. Ferritin is positioned as an inflammatory mediator and a consequence of this overexpression [87]. Ruscitti and colleagues [88] argue that ferritin and inflammatory cytokines interact in a complex and reciprocal manner. Additional inflammatory cytokines can be released as a result of ferritin's ability to start and intensify inflammatory cascades. The monocyte–macrophage system, an essential part of the inflammatory cytokine storm, is activated when there is an obvious increase in serum ferritin levels [88].

5.3 Ferritin in neurodevelopment and iron supply for neuronal growth and differentiation

Early-life iron deficiency can result in abnormalities in gene expression, neurotransmitter function, neurometabolism, and other aspects of brain development. These abnormalities can then impact children's sensorimotor functions, growth and development, cognitive language, social–emotional skills, learning and memory development, and other areas [89]. Iron is needed for brain development, enhancing neuronal maturation, formation of myelin, and neurotransmitter function. Iron is essential for the maturation of neurons. Ferritin levels have a favorable correlation with the sensorimotor, mesiotemporal, and source hypocortices' volumes. The risk of seizures can be increased by reduced peripheral iron and ferritin levels, which can potentially cause hypoplasia in the putamen and caudate nucleus [90]. According to an attention-deficit hyperactivity disorder (ADHD) study, children with ADHD had substantially lower blood ferritin levels than the control group [91]. More importantly, increased expression of FTMT has been shown to protect neuronal cells from degeneration by reducing the impact of ROS and promoting neuronal survival [92].

5.4 Ferritin in acute-phase reaction

Acute-phase reactants are inflammation markers that exhibit significant changes in the serum during inflammation, and there are positive and negative acute-phase reactants. Ferritin is one of the positive-phase reactants as it is upregulated during inflammation. To prevent microbial iron scavenging, ferritin sequesters iron. Ferritin levels are raised during infection and cancer to decrease the amount of free iron accessible to pathogens or tumor cells, respectively. Proinflammatory cytokines increase ferritin levels. Because they have virulence factors, siderophores (pyoverdine and pyocyanin) that chelate and import iron, some species, such as Pseudomonas, lower the ferritin levels [93].

6. Ferritin in pathological conditions

In various important cellular functions such as oxygen transport and exchange, ATP production, synthesis and repair of DNA, protection from oxidative damage, gene expression regulation, as well as amino acid and lipid metabolism, iron is required [94]. Approximately, in males, an average amount of iron is equivalent to 40 mg/kg of body weight, whereas in females it is 50 mg/kg. Serum ferritin concentration reflects the total body iron stores. In an apparently healthy individual,

1 ng/mL of serum ferritin indicates 8–10 mg of stored iron. An adequate serum ferritin threshold is essential for recognizing iron deficiency to avoid its progression to iron deficiency anemia and its preventable complications [95].

6.1 Iron deficiency and anemia

The level of serum ferritin closely correlates with total iron stores in the body, and the normal range is 30 to 300 ng/mL (67.4 to 674.1 pmol/L). Values at levels of less than 30 ng/mL [67.4 pmol/L]) They are specific to iron deficiency. However, ferritin has been reported as an acute-phase reactant, and the levels increase in inflammatory, infectious, and neoplastic disorders. These disorders may still be compatible with iron deficiency at a serum ferritin level up to 100 ng/mL.

One of the leading causes of global disease burden is iron deficiency, and it has a greater impact on infants, young children, women of reproductive age, as well as pregnant and postpartum women living in low- and middle-income countries [94].

Globally, iron deficiency has been labeled as the most common cause of anemia, and it has been linked to nutritional deficiencies. It has also been observed in patients with heart failure, chronic kidney disease, inflammatory bowel disease, and cancer. Iron deficiency has been associated with poor performance in daily activities, a reduction in the quality of life, frequent visits to the hospital, and death.

In the evaluation of iron stores, ferritin is used. In healthy individuals, absolute iron deficiency occurs at a decreased serum ferritin with a threshold of <30 μg/L, whereas at a threshold of <100 μg/L in chronic conditions, an inadequate level of iron reservoir is indicated. Although the threshold of serum ferritin concentration varies according to age, low ferritin levels are also observed in elderly patients due to iron reabsorption disorders or lower food intake [96].

6.2 Iron overload disorders

Iron is an essential metal found in all the cells of the body, playing an important role in many physiological and cellular processes. There are also many diseases associated with iron metabolism affecting many people. In particular, diseases of iron imbalance affect billions of patients, mostly with iron deficiency anemia [9]. Under normal physiologic conditions, iron concentration in the body is maintained at around 40 mg/kg in women and 50 mg/kg in men. About 80% of the body's iron is found within hemoglobin, myoglobin, and iron-containing enzymes, whereas the remaining 20% is found in ferritin, which is the primary iron storage protein. The primary mode of iron regulation is accomplished through modulation of absorption in the gastrointestinal tract in response to physiologic needs. Normally, approximately 10–20 mg of iron is ingested daily, of which only 10% or 1–2 mg is absorbed [97].

Ferritin has been known to play a crucial role in iron overload disorders (IODs). IODs are conditions that can lead to increased body iron stores and end-organ damage in affected organs. Increased iron depositions are commonly observed in the liver, heart, endocrine system, joints, and pancreas. IODs may be genetic or acquired, and elevated ferritin levels are a key indicator of IODs [98]. Iron overload can arise from chronic RBC transfusions, HH, ineffective erythropoiesis (IE), excessive iron supplementation, and dietary or environmental exposure to iron. These sources often lead to excessive iron accumulation in organs, that is, excessive ferritin buildup, causing toxicity and tissue damage [9].

IODs can manifest with heterogeneous clinical features: skin discoloration, arthritis, abdominal pain, infertility, impotence, cardiac arrhythmia, heart failure, new-onset or difficult-to-control diabetes mellitus, and elevation in liver enzymes [99].

6.2.1 Classification of iron overload

Iron overload can be classified into primary iron overload, which results from HH, and secondary iron overload results from iatrogenic iron administration, hematologic conditions that lead to IE or repeated packed RBC (PRBC) transfusions, and from liver disease [100].

Primary iron overload

Primary iron overload is most often inherited. HH is the leading cause of iron overload disease, and its clinical manifestation is systemic iron deposition mainly in the liver, heart, brain, and endocrine organs. In 1996, two gene mutations (C282Y and H63D) of the high iron (HFE) gene were discovered and linked to primary iron overload. The most common is C282Y, which encodes the HH protein (HFE protein) and plays a role in hepcidin regulation. Less common genetic mutations are found in hepcidin antimicrobial pepetide (HAMP), hemojuvelin (HJV), TFR2, and ferroportin gene (SLC40A1). These gene mutations are grouped as non-HFE hemochromatosis (McDowell & Sticco, 2024). Previously, hematochromatosis was classified into four types; Type 1 is classic hemochromatosis resulting from HFE gene mutation (C282Y or H63D). The remaining types are non-classical. Type 2A results from the HAMP mutation. Type 2B results from HJV mutation. Type 3 is due to a mutation in TFR2, and Type 4 is due to SLC40A1 (Crownover & Covey, 2013). The recent classification of hematochromatosis, which has the HFE-related, non-HFE-related, and digenic, has been proposed. The classification places type 1 into the HFE-related group. This also includes the heterozygosity of C282Y with other rare HFE variants. Groups Type 2, 3, and 4 fall into the non-HFE-related group. Digenic consists of double heterozygosity and/or double homozygosity/heterozygosity for mutations in two different genes, which can be HFE or non-HFE-related (Girelli et al., 2021).

Signs and symptoms of hemochromatosis include:

- Fatigue
- Feeling mentally drained and unable to concentrate
- Mood swing
- Weight loss
- Joint pain
- Weakness
- Signs of liver disease enlarged abdomen, darkening or gray-looking skin
- Diabetes due to damage to the pancreas

- Heart abnormalities irregular heart rhythms or heart failure
- Pituitary damage can cause menstrual irregularities or sexual dysfunction
- Damage to the adrenal gland can cause blood pressure abnormalities
- Damage to the thyroid gland can lead to hypothyroidism (reduced thyroid hormone) [98].

Secondary iron overload

Secondary iron overload is a consequence of a wide-spectrum of causes, including hematologic disorders causing IE, anemic conditions requiring repeated transfusion of RBCs, and chronic liver diseases unlike primary iron overload which is a well-recognized disease with available standard treatment recommendations. Secondary iron overload is more prevalent than primary iron overload as it is a more diverse diseasethat occurs as a consequence of various causes that differ significantly across geographic regions with many unclear areas to be explored [101].

Secondary iron overload encompasses the following etiologies:

- IE states affecting iron absorption or distribution, include thalassemias, sickle cell disease, sideroblastic anemias, chronic hemolytic anemia, pyruvate kinase deficiency, and dehydrated hereditary stomatocytosis.
- Iron overload secondary to excessive intake, including chronic or frequent RBC transfusions and inappropriate iron therapy (oral or intravenous (IV)).
- Miscellaneous causes: Porphyria cutanea tarda, aceruloplasminemia, congenital atransferrinemia [102].

6.2.2 Diagnosis

Initial biochemical testing, Transferrin saturation (TSAT) (>45% women, >50% men) is an early and sensitive marker. Serum ferritin reflects iron stores but can be raised by inflammation or liver disease; used with TSAT [98]. Fasting ferritin and transferrin saturation levels should be obtained in patients with chronic elevation in liver enzymes or those with unexplained signs and symptoms outlined previously. Targeted population-based screening for asymptomatic men of Northern European ancestry can also be considered [99, 103]. Ferritin, being an acute-phase reactant, can be elevated in response to processes that do not correlate with iron status, including inflammation, chronic disease, malignancy, and infection.

Magnetic resonance imaging (MRI) for liver iron quantification, MRI techniques (signal intensity ratio, T2 star (T2/T2*), Dixon-based) are now the primary non-invasive tools for measuring liver iron concentration (LIC), replacing biopsy due to accuracy, reproducibility, and safety advantages [97]. MRI can detect and assess iron overload in organs such as the liver, heart, spleen, and pancreas. On routine sequences, iron-rich tissues appear darker (hypointense) on T2-weighted and in-phase T1-weighted images. This pattern is seen regardless of whether the iron overload is primary hemochromatosis or secondary hemosiderosis, although marked pancreatic iron often points to primary hemochromatosis. Beyond qualitative assessment, MRI

can estimate tissue iron more precisely using gradient-echo (GRE) sequences and T2* values, with established formulae available to convert T2* into iron concentration in milligrams per gram of dry tissue [103]. Hepatic iron index on percutaneous liver biopsy can be utilized in difficult-to-diagnose cases but carries a greater procedural risk than blood testing. Liver biopsy was previously the standard for diagnosis, but with the advent of less invasive testing, it is now reserved for more complicated cases.

Finally, hepatic biopsy serves three main purposes: for diagnosis, disease staging, and to assist in therapeutic decision-making. Due to the close relationship between the iron and the liver (relating to total iron studies), the liver is the organ of choice for biopsies in the context of iron overload, where the appearance of iron-staining granules in periportal hepatocytes is the first evidence of overload. The histological staining of hepatic iron concentration is done by two methods: Biochemical methods, where techniques such as colorimetric and atomic absorption are employed, and semi-quantitative methods, where Prussian blue staining and different levels of microscopic magnification are assigned [104].

6.2.3 Treatment of IODs

Phlebotomy remains the first-line therapy for patients with HH who are not anemic. Regular venesection effectively depletes iron stores by inducing erythropoiesis. Phlebotomy encompasses an induction and maintenance phase. During the induction phase, phlebotomy should be performed weekly (or fortnightly) until iron stores are depleted. The target for iron depletion during induction is a serum ferritin not lower than 50 μg/L to avoid iron deficiency. In the maintenance phase, serum ferritin can be maintained with some flexibility in the range of 50–100 μg/L [105].

In transfusion-dependent patients, iron chelation therapy is the cornerstone of treatment. Iron chelation therapy is used to reduce the development of iron overload due to its deposition in various organs, such as the liver and heart, after regular transfusion. Chelation therapy aims to prevent the accumulation of excess iron and its complications, such as hepatic, endocrinological, and cardiac dysfunction [106].

Recent research focuses on modulating iron homeostasis pathways: Hepcidin agonists and mimetics. Hepcidin is a key regulator of iron metabolism. Agents like rusfertide (a hepcidin mimetic) show promise in controlling erythrocytosis and iron overload in clinical trials [107].

6.3 Inflammatory diseases

Ferritin is a positive acute-phase reactant, and the concentration is elevated during inflammation. Ferritin acts by sequestering iron to inhibit microbial iron scavenging. In a case of malignancy and infection, the concentrations of ferritin are increased to reduce the availability of iron to tumor cells or the causative pathogen, respectively. Its roles are enhanced by proinflammatory cytokines such as IL-6, IL-1β, as well as tumor necrosis factor-alpha [93, 108]. Evidence shows that ferritin is not only an acute-phase reactant but also an active trigger of the inflammatory process. This pro-inflammatory activity of ferritin involves multiple receptors in ferritin uptake, such as TfR1. The process also activates NF-κB, resulting in increased inflammatory cytokine expression. Ferritin can also function as a damage-associated molecular pattern (DAMP), which can activate a multi-protein complex –

inflammasome, which is a NOD-like receptor protein 3, (NLRP3), which is essential to the innate immune response that promotes the maturation and release of pro-inflammatory cytokines [44].

6.4 Neurodegenerative disorders

Ferritin stores iron needed for body metabolism and releases it when necessary. For instance, ferritin-liberated iron is an essential trace element that is involved in numerous physiological processes such as transportation of oxygen to brain tissue, DNA synthesis, energy metabolism, myelin synthesis, as well as neurotransmitter production. Ferritin dysregulation and alteration in iron homeostasis, either by deficiency or excess, can affect normal neurological function. Inadequate iron levels are commonly seen in premature infants and infants with placental dysfunction or in newborns who develop postpartum hemorrhage, and these conditions might increase the risk of iron deficiency due to the rapid development of the brain. When there is inadequate iron supplementation, impaired brain development ensues, which will adversely affect intelligence and social skills [90]. Also, dysregulation of ferritin that may cause excessive iron accumulation in the brain tissue, which may be due to the aging process, may leave individuals susceptible to neurological disease due to triggered oxidative stress and neural cell damage [109]. These neurological diseases, including Alzheimer's disease (AD), Parkinson's disease (PD), multiple sclerosis (MS), amyotrophic lateral sclerosis (ALS), and Huntington's disease (HD), are characterized by the progressive loss of neurons or myelin in both the brain and spinal cord [110]. Mutation of the ferritin light chain may cause inclusion bodies in neuronal and glial cells, contributing to neural disease development and progression [111].

6.5 Cancers

Ferritin has been reported by several studies to be implicated in the development and progression of many forms of cancer. The correlation between ferritin, tumors, tumor microenvironment, cancer, and metastasis is a complex one and may vary according to cancer subtype. Unmodified human FTH1 is significantly and positively correlated with the infiltration of tumor-associated macrophages [112]. The tumor microenvironment is a complex condition that uses several mechanisms of ferritin gene expression, which it does by targeting DNA and RNA regulatory elements. Also, FTH1 protects cancer cells from hypoxia damage while promoting and sustaining tumorigenesis. FTH reduces the level of ROS, thereby increasing chemoresistance in cancers [35]. In breast, ovarian, pancreatic, colorectal, and lung cancers, elevated ferritin levels have been noted and have served as prognostic factors. Hyperferritinemia has been reported in cancer and has been suggested as a tumor marker in the diagnosis of malignancies [113]. Therapeutically, cytoplasmic FTH1 has been found to play a role in the regulation of MHC1, which helps to present tumor antigens for destruction and attracts CD8+ cells for that purpose [114].

7. Clinical implications, future directions, and emerging research

The role of ferritin in iron metabolism makes it a clinically significant biomarker and therapeutic target. Its dynamic regulation reflects diverse physiological states

from iron overload and deficiency to inflammation, infection, and malignancy. Understanding the implications of altered ferritin expression is essential for diagnosing and managing a broad range of clinical conditions. Ferritin has been discussed extensively for its role in iron release and storage as well as its interaction with other proteins of iron metabolism, while noting its role in physiological and pathological conditions, yet there are unresolved questions concerning ferritin and its mechanism of involvement in iron release and non-iron-related cellular processes.

7.1 Unresolved questions about mechanisms of iron release from ferritin

Although the role of ferritin in iron storage is well defined, the mechanisms by which iron is released remain unclear. Iron within ferritin is stored as a ferrihydrite-like mineral core, which is known to exist in a solubilized state and reduced form transitioning from insoluble form Fe^{3+} to the Fe^{2+} form before cellular utilization [115]. Several potential mechanisms have been proposed, including chelation by reductants (such as flavins or NADPH), direct electron transfer via reductases (e.g., STEAP3), and lysosomal degradation during ferritinophagy [116]. However, the physiological relevance of each pathway remains to be fully delineated. For instance, does reductive mobilization predominate under basal conditions, while lysosomal degradation is activated during iron starvation or inflammation? Additionally, the molecular regulators of these pathways, such as iron transporters [DMT1, Zrt and Irt-like protein 14 (ZIP14)], autophagy-related proteins (NCOA4), and ROS, require further clarification [117]. Understanding these mechanisms could reveal therapeutic targets for diseases of iron mismanagement, such as neurodegeneration, anemia of chronic disease, and cancer.

7.2 Ferritin's role in non-iron-related cellular processes

Emerging evidence suggests that ferritin may influence cellular processes unrelated to iron metabolism. For instance, FTH1 is implicated in the regulation of immune responses, oxidative stress mitigation, and tumor progression. Extracellular ferritin, which can circulate in plasma or be secreted by macrophages, appears to function as an immunomodulatory molecule, interacting with cell surface receptors such as T cell immunoglobulin and mucin domain-containing protein 2 (TIM-2), SCARA5, and TfR1. Ferritin may also impact angiogenesis, mitochondrial function, and stem cell maintenance [118–120]. Despite these discoveries, the mechanistic basis of these roles remains largely speculative as to whether these effects are iron-dependent or independent. Does the source of ferritin (intracellular vs. extracellular, heavy vs. light chain composition) influence its function? The heterogeneity of ferritin isoforms and their post-translational modifications could hold the key to these functional differences. Future research should aim to characterize these variants in different tissues and disease contexts, using proteomic tools.

7.3 Technological advances; high-resolution structural studies

Recent breakthroughs in cryo-electron microscopy (cryo-EM), X-ray crystallography, and nuclear magnetic resonance (NMR) spectroscopy offer unprecedented opportunities to resolve the structure of ferritin and its complexes at atomic resolution. Detailed visualization of the ferroxidase center, threefold

and fourfold channels, and mineral core has enhanced our understanding of iron entry, oxidation, and mineralization [121, 122]. However, dynamic aspects of ferritin, such as transient conformational changes during iron loading/unloading or interactions with partner proteins (e.g., NCOA4, STEAP3), remain poorly visualized. Integrative approaches combining cryo-EM with molecular dynamics and simulations or real-time imaging (e.g., flourescence resonance energy transfer [FRET] or atomic force microscopy [AFM]) could provide more insights into these events. Moreover, the structural differences between ferritin chains (FTH1, FTL), FTMT, and pathogen-associated ferritins (bacterial and archaeal types) also require further investigation. Structural insights into these variants may inform the development of isoform-specific modulators, aiding therapeutic selectivity in targeting ferritin.

The invention of single-cell RNA sequencing (scRNA-seq), mass cytometry, and spatial transcriptomics has revolutionized the understanding of cellular heterogeneity. These techniques could help uncover cell type, expression patterns, regulatory mechanisms, and the exact physiological and pathological response of ferritin to stress. Techniques like scRNA-seq could reveal how ferritin gene expression varies in tumor microenvironments, across immune cell subsets, or in neurodegenerative lesions [123, 124]. Utilizing two techniques, such as transcriptomics with single-cell proteomics, allows for the parallel measurement of FTH1, FTL, and FTMT at the protein level. This is specifically relevant given the post-transcriptional regulation of ferritin translation through the IRE/IRP system. In addition, emerging tools such as single-molecule fluorescence *in situ* hybridization (FISH) or proximity ligation assays (PLA) can be used for ferritin localization and its interaction with other molecules on a subcellular level, offering insights into compartmentalized functions (e.g., nuclear ferritin, mitochondrial FTMT).

7.4 Translational research (bridging basic science and clinical applications)

To bridge the gap between research and medicine is a major challenge, but could be an opportunity if turned into clinically actionable knowledge. In diseases such as hemochromatosis, anemia of chronic disease, sideroblastic anemias, and neurodegenerative disease, altered ferritin levels are common, yet their diagnostic and prognostic significance remains clinically underutilized, and hence, it is not explored as a therapeutic option in these diseases. Similarly, in cancer, ferritin is increasingly recognized not merely as a marker but as a modulator of tumor immunity, angiogenesis, and ferroptosis. Clinical research should focus on defining ferritin signatures across disease states to help define how it can be used diagnostically or therapeutically. For instance, determine the ratio of the ferritin chains and how it affects the disease prognosis, or can this ratio predict prognosis or therapeutic response in cancer? Integrating ferritin measurements into multiparametric diagnostic panels could improve specificity in differential diagnoses, especially in conditions with overlapping inflammatory and iron-loading features. Furthermore, preclinical studies in animal models should evaluate the consequences of genetic or pharmacological modulation of ferritin pathways, such as NCOA4 deletion (blocking ferritinophagy) or TfR1 overexpression (altering ferritin uptake). Such studies will clarify therapeutic feasibility and potential side effects of targeting ferritin.

7.5 Personalized medicine approaches targeting ferritin pathways

Personalized medicine promises tailored interventions based on individual genetic, molecular, and environmental factors. For instance, in cancer, many tumors overexpress ferritin to support rapid proliferation and evade ferroptosis. Targeting ferritin or its regulators (e.g., NCOA4, ferroptosis suppressor protein 1[FSP1], Glutathione Peroxidase 4 [GPX4]) may sensitize tumors to iron-induced cell death. Ferritin-targeted drug delivery systems leveraging ferritin nanocages to encapsulate chemotherapeutics are being explored for enhanced tumor selectivity and reduced toxicity. In PD and AD, iron accumulation and ferritin dysregulation contribute to oxidative stress and neuronal death. Biomarkers based on ferritin isoforms in cerebrospinal fluid or plasma could guide early diagnosis and stratification of patients for antioxidant or chelation therapy [125]. Inflammatory disorders such as systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), and COVID-19, hyperferritinemia is associated with macrophage activation and cytokine storms. Understanding the mechanistic role of extracellular ferritin in immune modulation could enable targeted anti-inflammatory strategies, such as blocking ferritin receptors or neutralizing pro-inflammatory ferritin complexes. To realize these goals, integrative efforts are needed to characterize ferritin-regulated networks across populations, incorporating genomics, transcriptomics, proteomics, and metabolomics. Clinical trials should evaluate not only efficacy but also biomarkers of target engagement and patient stratification.

8. Conclusion

Ferritin biology sits at the core of iron metabolism, oxidative stress, immunity, and cellular homeostasis. The regulation and expression of ferritin through the transcriptional, translational, and post-translational modification mechanisms play a huge role in releasing iron for cellular activities while preventing overload that may cause cellular toxicity. Ferritin dysregulation can lead to a wide array of diseases, such as anemia, iron overload, neurodevelopmental and neurodegenerative pathologies, and most of all has been seen to drive inflammation in several cancers, thereby worsening cancer prognosis. While decades of research have highlighted many of its foundational roles in health and disease, it has also opened avenues for research on ferritin for use as a targeted therapeutic intervention for iron-related and non-iron-related pathologies. It's use as a nano-transporter is also being explored currently. Despite all this information and advances, critical knowledge gaps remain. Unlocking the secrets of ferritin's roles outside its classical cellular roles will require the convergence of cutting-edge technologies, interdisciplinary collaboration, and translational vision. Although ferritin remains the master regulator of iron metabolism, advances in its structural biology, elucidate its function at unprecedented resolution. So far the activities of ferritin within the terrain of iron homeostasis have been well understood, yet ongoing research is important to futher characterize its role in pathologies of clinical concern, especially in neuroscience and oncology. As research continues to build up in the area of precision and personalized medicine, ferritin and its regulatory pathways may play a crucial role as diagnostic and prognostic biomarkers and may also be useful therapeutic targets in diverse clinical contexts such as fields of oncology,neurology, and immunology. The future

of ferritin research lies not only in answering existing questions but also in revisiting its place in cellular physiology. This paradigm shift from a static iron depot to a dynamic regulator promises to open new frontiers in biomedical science.

Conflict of interest

The authors declare no conflict of interest.

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Chapter 3

Normal Aspects in Blood Transfusion: From Donor to Patient

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Abstract

Blood transfusion is a process where the donated blood or blood components are given through an intravenous line if a person has lost blood through an injury, surgery, or a severe medical condition that affects the blood or its components. The main aim of blood transfusion is to optimize the level of hemoglobin. It also helps in various medical conditions like anemia, cancers, hemophilia, blood loss, etc. ABO blood grouping system and Rh blood grouping system play an important and reliable blood grouping for blood transfusion. Many of the physiological aspects of blood transfusion are intravascular volume and increase in blood pressure. For safe and easily accessible blood products, sufficient blood banks, qualified personnel, and appropriate storage facilities are required. Transfusion-transmitted infections (TTI) are reduced due to stringent donor selection and screening procedures with the help of various technologies to test donors' blood for infections and routine surveillance of regulatory authorities of blood supply chains. The downside of blood transfusion comprises of blood transfusion reaction which might occur 24 hours after an infusion spanning from mild to severe and life-threatening risks. Hence, safety improvement leads to lower risk in transfusion medicine and therefore systemic screening tests are considered to reduce potential pathogen load in blood transfusion.

Keywords: blood transfusion, blood grouping, transfusion-transmitted infections, screening test, risks and safety precautions

1. Introduction

Blood transfusion is a process of transferring blood from a healthy individual to the patient's body. The transfusion of blood is carried out to increase arterial oxygen levels and transport oxygen to tissues. Blood transfusions are effective in many medical surgeries [1]. Whole blood transfusion involves the transfer of all the blood components containing red blood cells(RBCs), white blood cells(WBCs), platelets,

and plasma from the donor in severe cases such as traumatic hemorrhage [2]. On the other hand, allogeneic red blood transfusion involves the transfer of a specific part of a blood component from the donor to the recipient under critical conditions, for example, the transfusion of RBCs in anemia, plasma in blood clots and platelets in case of thrombocytopenia [1, 3].

Safe blood transfusion is therefore based on accurate blood grouping. Blood group testing and cross-matching are important tests done during surgical operations [4]. Blood grouping is of two types ABO method and rhesus(Rh) method. Mixing of blood groups leads to blood clumping or agglutination. This causes hemolytic transfusion reaction and hemolytic disease in the newborn. To avoid these conditions, it is important to match the blood group correctly [5]. If the blood group is incorrectly matched, this condition is known as ABO incompatibility. ABO incompatibility is one of the main causes of hemolysis. It occurs when a person receives a different blood type from the donor. For example, a person with O type blood receiving A, B, or AB type blood group from the donor experience ABO incompatibility [6, 7]. An adverse transfusion reaction(ATR) is a harmful response to a blood transfusion that ranges from mild to severe. These reactions can be immediate or delayed and can be caused by immune or nonimmune factors [8, 9].

The correct matching of blood groups reduces the impact of transfusion-transmitted infections (TTI) such as HIV and hepatitis [10]. Transfusion-transmitted infection is transferred from the donor's blood to the recipient's blood through blood transfusion if the donor's blood is contaminated. This infection may be caused by bacteria, viruses, parasites, prions, pathogens, etc. [11]. The preventive measures for this infection are faster and more effective, followed by screening methods [12]. Screening of blood is a method used to prevent TTI. Screening of donor's blood to check for any infectious disease includes predonation and postdonation testing of blood products [13]. Rapid diagnostic screening transfusion is also used in the areas of low facilities and for quick diagnosis of specific infections using a small number of samples [14]. Therefore, the screening method is used to ensure the safety of both donor and patient before blood transfusion.

This chapter deals with the mechanism and importance of blood transfusion from donor to patient, different blood grouping systems accounting for its compatibility, adverse transfusion reactions, transfusion-transmitted infections, screening tests for blood transfusion, safety precautions for blood transfusion, and challenges.

2. Blood grouping system

Animal-to-animal blood transfusion was first performed in 1665 by Richard Lower, and later animal-to-human blood transfusion by Jean-Baptiste Denis in 1667. British obstetrician James Blundell carried out the first human-to-human blood transfusion in 1818 [2, 15]. Blood groups are determined by the presence of antigens on the surface of RBCs, mainly the ABO blood group system and the Rh blood group system. Accurate blood typing is essential before a blood transfusion to avoid issues [16]. Clinically significant antibodies cause adverse reactions after blood transfusion and lead to hemolytic disease in newborns through placental transfer from mother to fetus [17]. There are around 33 blood group systems that represent over 300 antigens. The antigens are integral proteins where polymorphisms are present in the variation of amino acid sequence (e.g., rhesus [Rh] and Kell), glycoproteins, or glycolipids (e.g., ABO) [4]. ABO incompatibility is one condition where blood transfusions occur

due to error [7]. The signs and symptoms include Hypotension, hemoglobinuria, and hemoglobinemia [18]. For blood transfusions, the O-negative blood group is the universal donor, while the AB-positive blood group is the universal recipient. Incorrect matching in the ABO system can cause severe immune responses, such as hemolysis [19]. Delayed-type hemolytic transfusion reactions (DHTR) is a condition that occurs when the immune system destroys transfused red blood cells [20]. ABO and Rh blood grouping are further seen in detail.

2.1 ABO blood grouping

Among all the blood grouping systems, ABO is the most important in blood transfusion [4]. It is categorized into A, B, AB, or O based on the antigen present on the surface of RBCs. Group A encodes for N-acetyl D-galactosamine, group B for D-galactose amine, group AB for both, and group O for no antigens. The plasma contains the antibodies against these antigens for example, anti-B antibodies in group A and both anti-A and anti-B antibodies in group O [21]. The ABO blood group system is important for blood transfusions, as mismatched blood can cause adverse reactions like hemolysis [22]. The O-negative blood group is considered as a universal donor and AB-positive individuals are universal recipients [23]. This blood group system also plays a role in organ transplantation and ensures compatibility to minimize the risk of rejection [24]. ABO incompatibility between a mother and fetus causes mild hemolytic disease in the newborn (HDN) [25, 26]. ABO blood grouping is simple, involving serological methods that detect antigen-antibody reactions [27]. This antibody-antigen interaction is essential for ensuring compatibility in blood transfusions, as mismatched transfusions can lead to severe immune reactions like hemolysis [28].

2.2 Rh blood grouping

The Rh blood grouping was discovered by Landsteiner and Weiner in 1941 [21]. The Rh blood group system is more diverse and complex and consists of many variations, which make it one of the most immunologically significant systems in humans. This plays a major role in blood transfusions [29]. It is the second most important system after ABO in blood grouping. The Rh system includes 50 identified blood group antigens, but only five are considered clinically significant due to their major role in blood transfusion and pregnancy-related complications. The five important Rh antigens are D, C, c, E, and e [4]. Once the D-positive red blood cells are transfused into D-negative individuals, about 80% develop anti-D antibodies. This anti-D response triggers the production of antibodies against other blood group antigens, both within and beyond the Rh system [30]. According to Fisher and Race's theory, the Rh system consists of three closely linked genes, each with two alleles: C and c, D and d, and E and e. Individuals inherit one set of these alleles, called haplotypes, from each parent [31]. Differentiating RHD alleles that produce weak D and partial D antigens is clinically significant for assessing the risk of alloimmunization and their impact on blood transfusion management [32].

3. Mechanism and importance of blood transfusion

Blood includes red cell concentrates, platelet concentrates, and plasma. They can be obtained, either by the processing of whole blood donations or by apheresis. Separation of whole blood usually uses centrifugation, with two primary protocols

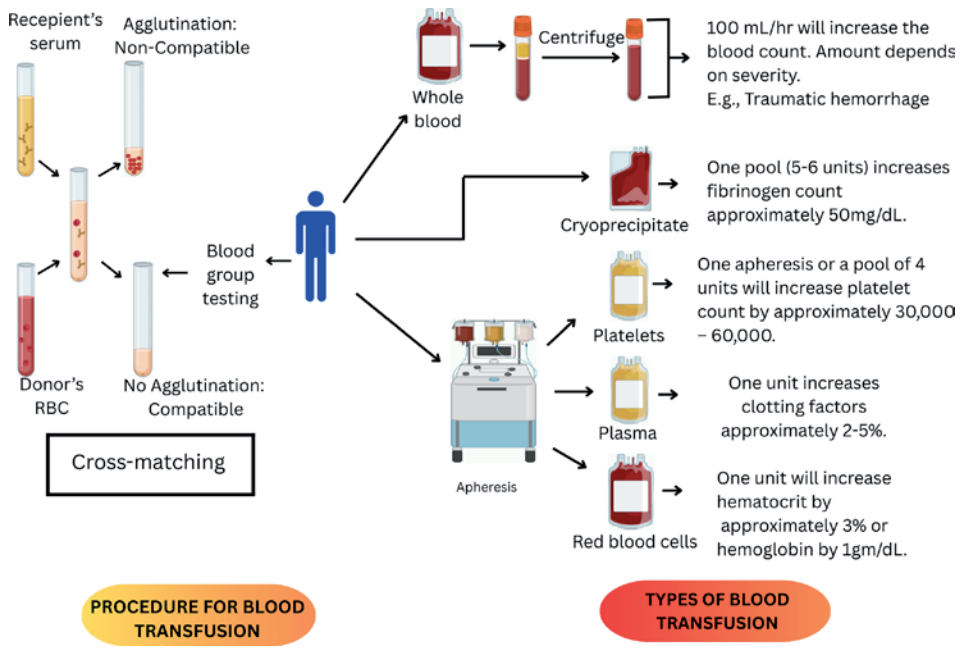


Figure 1.
The figure depicts the procedure to be followed before blood transfusion and the types of blood transfusion, along with the quantity required to be transfused.

resulting in two different intermediate products, namely platelet-rich plasma (PRP) and buffy coat (BC). Each blood component has unique storage conditions: plasma is frozen, and red blood cells and platelets are refrigerated or kept at controlled room temperature [33]. The ABO blood grouping system categorizes blood into four groups based on the presence or absence of antigens on the red blood cells: those with the A antigen (Group A), those with the B antigen (Group B), those with both A and B antigens (group AB) or those with neither antigen (Group O). Individuals also develop antibodies, such as anti-A or anti-B antibodies, based on their blood group [34].

Blood transfusion is one of the most widely utilized treatments globally, offering significant benefits but also carrying associated risks and costs [35]. It remains a cornerstone in managing trauma-induced coagulopathy [36]. Transfusions are a critical, at times life-saving, therapy for equine patients with acute hemorrhage and other causes of anemia. Transfusions enhance oxygen delivery to tissues both by increasing blood volume and by elevating hemoglobin concentration. RBC transfusions are widely used in intensive care units (ICUs) [37]. Despite the performance of many clinical randomized trials determining hemoglobin (Hb) levels for transfusion, there remains a lack of information on whether these guidelines translate into everyday decision-making in a veterinary clinical setting (**Figure 1**) [38].

4. Transfusion-transmitted infections (TTI)

Transfusion-transmitted infections, though rare, remain a notable risk with allogenic blood transfusions [39]. They are particularly concerning for individuals requiring multiple transfusions, such as solid organ transplant (SOT) recipients

or those with weakened immune systems [40]. HIV-positive individuals are especially vulnerable due to their compromised immunity, which increases their risk of acquiring additional infections from transfused blood [41]. While parasitic infections are less common than bacterial or viral ones, they can still lead to serious illness, especially in immunocompromised patients. Common parasites linked to these infections include *Plasmodium* species, *Trypanosoma cruzi*, *Babesia microti*, *Toxoplasma gondii*, and *Leishmania* species [12]. The non-uniform screening of parasitic infections is the major difficulty in diagnosing these parasites. In poorer areas, there is an added risk due to a lack of infrastructure and inadequately sensitive detection techniques [12, 41].

The safety measures concerning blood should include improved donor deferral criteria and universal nucleic acid testing (NAT) for key pathogens along with pathogen reduction technologies [42]. In this regard, hemovigilance systems, along with region-specific targeted screening of endemic parasites, are equal necessities. This further enhances the safety of transfusion as healthcare provider awareness improves and the education of the donor about the risks involved is enhanced [43].

5. Screening test for blood transfusion

Screening tests for detecting various infections that are major concerns worldwide are well established. Common infections detected using screening tests are hepatitis B virus (HBV), hepatitis C virus (HCV), human immunodeficiency virus, and syphilis [44]. Screening of blood donors began in the year of 1940s for testing syphilis.

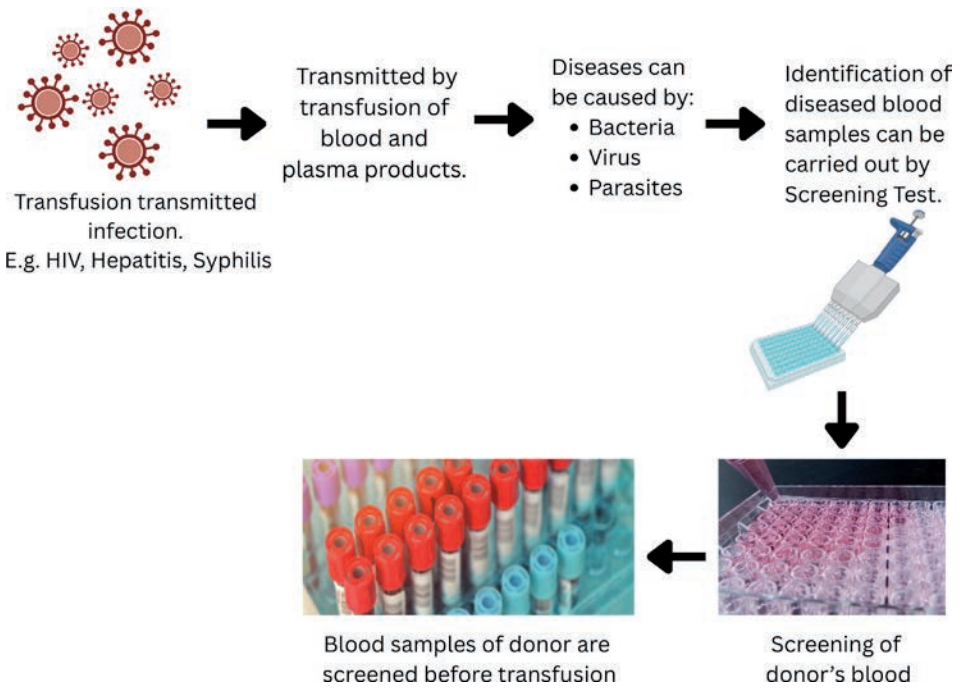


Figure 2.
Methods involved in the screening of donor's blood to check for any infection in the sample before transfusion to avoid transfusion-transmitted infection.

Hepatitis B surface antigen was first screened in the early 1970s [12]. During the early 1970s, people who had multiple blood transfusions had transfusion-transmitted HBV. In some countries, only HbsAg and HBV DNA tests are performed for hepatitis B [45]. HbsAg is the earliest marker of HBV infection and is the first test for HBV screening in blood donors. HBsAg screening needs high sensitivity to shorten the window period, the time of infection to antigen detection and to identify low HbsAg levels in the asymptomatic stage of chronic infection [46]. Hepatitis C was recognized with the help of an antibody test with which transfusion-transmitted HCV sharply declined. HCV antibody tests were introduced as blood-donation screening tests in the early 1990s [47]. Each blood donation undergoes multiple tests to identify the transfusion transmittable infections. All these tests detect antigens, antibodies, or nucleic acids of the infectious agents (**Figure 2**) [48].

6. Risks in blood transfusion

Blood transfusion has several risks ranging from mild to severe. There are infectious and non-infectious risks to patients, although the safety of blood transfusion has improved. To reduce the risk of blood transfusion, various blood conservation strategies should be utilized [49]. The major risks in blood transfusion include alloimmunization, febrile non-hemolytic transfusion reactions (NHTR), allergic reactions, Transfusion-related acute lung injury (TRALI), Transfusion-associated circulatory overload (TACO), and hemolytic transfusion reactions (HTRs) due to incompatibility [50]. NHTR, TACO, and TRALI cause symptoms like fever, chills, respiratory disease, and hemodynamic instability [51]. TRALI is commonly associated with donor antibodies or lipid mediators attacking pulmonary leukocytes, whereas TACO is commonly associated with fluid overload, particularly in elderly patients or in those with pre-existing cardiac disease [52]. Blood transfusion also carries significant risks in the transmission of infectious organisms such as viral or bacterial agents which has the most complications [53]. Although infections by infectious agents are infrequent, largely due to the strict methods of screening in place, they may involve viral contamination, such as HIV or hepatitis B/C, and bacterial contamination, more significantly with platelets stored at room temperature. The bacteria may cause sepsis-a potentially fatal condition with a mortality rate of up to 26% [54]. Proper matching, screening, monitoring, and prevention strategies are essential to minimize these risks.

7. Safety precautions during blood transfusion

Hospitals everywhere should follow proper safety protocols, and transfusion policies to have a clear view of the identification of the patient, and safely administer the blood components. All the hospital staff involved in the blood transfusion process, phlebotomists, porters, and medical and nursing staff, must undergo training and competency training [55]. Some strategies for enhancing safety precautions by reducing allogenic transfusions are: Avoiding pooled blood products reduces the number of exposures to allogenic blood donors; if it is not avoided, it can lead to death by TTI. The use of Fresh Frozen plasma and single donor platelets directly from either a male donor or female donor that does not have a history of pregnancy in the case

of females, if it is not prevented, can lead to transfusion-related lung infections [52]. Patient blood sample management approaches by implementing good transfusion practices greatly reduce transfusion rates [56]. An unsafe supply of blood is costly for both humans and the economy. Transfusion of infected blood samples causes mortality and morbidity in the recipient. Infections by blood transfusion can spread through a wider range. So, necessary precautions must be taken. Selecting low-risk blood donors is necessary [57]. Widespread causes of major hemolytic transfusion are improper administrative methods and improper identification of the donor [58].

8. Conclusion

Blood transfusion is an important medical procedure that saves lives by replacing blood components in patients with blood loss, anemia, or other severe conditions. There are risks such as febrile non-hemolytic transfusion reactions (NHTR), transfusion-related acute lung injury (TRALI), transfusion-associated circulatory overload (TACO), and hemolytic transfusion reactions (HTRs) that can result in symptoms like fever, respiratory disease, and hemodynamic instability. Although rare because of strict screening, infectious risks are due to transmission of viral agents like HIV and hepatitis B/C and bacterial contamination, especially of platelets stored at room temperature. Using donor screening, nucleic acid testing, leukoreduction, and pathogen reduction technologies has greatly decreased these risks. The need to ensure safety emphasizes proper patient identification, transfusion protocols, staff training, and the adoption of blood conservation strategies and hemovigilance systems. With the advancement of screening technologies, it is critical to continue improvement and follow stringent safety protocols strictly to maximize benefits from transfusion with minimal potential for complications. Advancements in blood transfusion science, with donor screening, improved, and pathogen reduction technologies, have significantly increased and enhanced the safety and efficacy. However, challenges keep persisting globally, like unequal access to blood products, regulatory gaps, and vulnerable crises like the COVID-19 pandemic, and disrupted blood donation systems. To address the problems, better strategies are needed, including strong regulatory frameworks, donation campaigns, and implementation of protocols. Accessibility is essential to ensure that blood transfusions are met at high safety standards.

Abbreviations

TTI	transfusion-transmitted infections
RBCs	red blood cells
WBCs	white blood cells
ATR	adverse transfusion reaction
DHTR	delayed-type hemolytic transfusion reactions
HDN	hemolytic disease of the newborn
PRP	platelet-rich plasma
BC	buffy coat

ICUs	intensive care units
Hb	hemoglobin
SOT	solid organ transplant
NAT	nucleic acid testing
HBV	hepatitis B virus
HCV	hepatitis C virus
NHTR	non-hemolytic transfusion reactions
TRALI	transfusion-related acute lung injury
TACO	transfusion-associated circulatory overload
HTRs	hemolytic transfusion reactions

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Chapter 4

Iron Deficiency and Hypothyroidism

Vijayakumar Savitha

Abstract

Thyroid diseases have become very common nowadays. Hypothyroidism is increasing in prevalence, with the most common cause being autoimmune conditions, followed by iodine deficiency, thyroiditis, drugs, and other secondary causes. Recently, deficiency of micronutrients like iron, selenium, and zinc has shown an association with hypothyroidism. I will be elaborating on the association between iron deficiency and hypothyroidism, as well as the mechanisms by which iron deficiency can lead to hypothyroidism, in this chapter.

Keywords: hypothyroidism, iron deficiency, pregnancy, TPO antibodies, TPO enzyme

1. Introduction

Thyroid disorders are increasingly prevalent worldwide, with hypothyroidism emerging as a common clinical entity. While autoimmune thyroiditis remains the leading cause, other contributors include iodine deficiency, subacute or silent thyroiditis, certain medications, and secondary endocrine or systemic conditions. In recent years, growing attention has been directed toward the role of micronutrient deficiencies – particularly iron, selenium, and zinc – in the pathophysiology of thyroid dysfunction. Among these, iron deficiency (ID) has been increasingly implicated as a potential modifiable factor influencing thyroid hormone metabolism. This chapter will explore the association between ID and hypothyroidism, with a focus on the underlying biological mechanisms and clinical implications of this relationship.

2. Epidemiology

2.1 Global burden of hypothyroidism

Hypothyroidism is a common endocrine disorder whose measured prevalence varies substantially by geographic region, iodine intake, age distribution, sex, and case-finding intensity. Population prevalence estimates of overt hypothyroidism in iodine-replete populations typically range from approximately 1–2%, whereas

estimates that include subclinical hypothyroidism commonly cite an overall population prevalence of approximately 4–10%. These global estimates reflect a mixture of screening studies, community surveys, and health-service data and are sensitive to assay cutoffs and the intensity of testing in a given health system [1, 2].

There is clear sex and age stratification: women are affected much more commonly than men (female-to-male ratios commonly reported between 3 and 8:1), and prevalence increases with age, particularly after the fifth decade. Changes in diagnostic thresholds and more sensitive TSH assays have increased the number of people labeled with "mild" or subclinical thyroid dysfunction in many high-income settings, while in low and middle-income countries (LMICs), iodine deficiency and goiter still contribute significantly to hypothyroidism in certain regions [2].

Country-level and regional heterogeneity is marked. Large Indian population surveys, for example, have reported substantially higher community prevalences than many Western cohorts: a multicity epidemiological survey in India found an overall prevalence of hypothyroidism of approximately 11% (10.95%) across eight cities, with marked inter-city variability (for example, Kolkata >20% in that sample). Subclinical hypothyroidism is even more common, with prevalence ranging from 8–20%, depending on population characteristics and assay cutoffs [3].

In our study conducted in Mysuru, India, where the sample size was 500 pregnant women, the prevalence of hypothyroidism in first-trimester pregnant women was 156/491 (31.77%). Of this, 9 (5.76%) had overt hypothyroidism and 147 (94.23%) had subclinical hypothyroidism, while 7 (1.42%) had hyperthyroidism [4]. These figures likely reflect true regional variation (iodine status, genetic and autoimmune disease prevalence), differences in case detection, and population structure [5].

Global burden datasets (GBD) and recent systematic reviews emphasize that, while age-standardized rates of clinically severe hypothyroidism have changed little in some regions, the absolute number of people living with thyroid dysfunction has risen, driven mainly by population aging, improved diagnosis, and persistent pockets of iodine insufficiency in parts of the world. This matters: even subclinical disease carries implications for quality of life, cardiovascular risk, and, in pregnancy, neurodevelopmental outcomes in offspring – hence the epidemiological and public-health attention [6].

Key recent reviews that synthesize these data and unpack methodological heterogeneity include Vanderpump's epidemiology summary and the Nature Reviews Endocrinology review on global hyper and hypothyroidism as shown in **Table 1**;

Region/country	Hypothyroidism (overt + subclinical)	Key source
India	10.95% (population survey)	[5]
United States of America	≈9.6% (NHANES 2009–2012 estimate/administrative datasets suggest 9.5–11.7%)	(NHANES/Wyne 2022)
China	13.95% (large cross-sectional 31-province study)	(Li et al., 2023)
Europe (pooled)	Total ~4.7% (meta-analysis of undiagnosed hypothyroidism)	(Mendes et al., 2019)
Sub-Saharan Africa (region)	Variable; limited representative data – likely lower detection and wide heterogeneity	[2] (Fan 2023)

Table 1.
Hypothyroidism prevalence – selected countries/regions.

these are useful resources when contextualizing prevalence numbers and comparing study methodologies [1, 2].

2.2 Global burden of iron deficiency

ID (ID) and iron deficiency anemia (IDA) remain the most common micronutrient disorders worldwide. The World Health Organization's global estimates and recent large meta-analyses indicate that hundreds of millions of people – especially women of reproductive age, pregnant women, and young children – are affected. For example, WHO and related global reports estimate that approximately 30–40% of pregnant women and about one-third of nonpregnant women worldwide have some degree of anemia, much of which is attributable to ID in settings without high rates of hemoglobinopathies or infectious causes [7].

Country-level surveillance highlights the striking burden in South Asia and Sub-Saharan Africa. India's large, nationally representative National Family Health Survey (NFHS-5, 2019–21) documents persistently high anemia prevalence among women of reproductive age as shown in **Table 2**. NFHS-5 reports that over half of women aged 15–49 years are anemic (estimates vary by pregnancy status and state) [8]. In an Indian study, 26.1% of pregnant women were anemic, primarily classified as mild. ID was observed in 61.4% of subjects, with 28.1% having serum ferritin levels below 15 ng/mL [9].

Beyond hemoglobin estimates, iron-status biomarkers show widespread low ferritin concentrations in pregnant cohorts in LMICs. Many hospital and community-based studies report ferritin <15 ng/mL in a large fraction of pregnant women (often >25–30% depending on the cohort and trimester), with ID contributing to maternal morbidity and adverse perinatal outcomes. The high

Region/country	Anemia prevalence (women 15–49)	Key source	Anemia prevalence (pregnancy)
India (WRA, NFHS-5)	≈53% (national NFHS-5 aggregated estimate; varies by state)	(NFHS-5); [11]	
United States (WRA, NHANES)	≈5.6% (NHANES 2003–2012 average); more recent CDC briefs show higher age-specific estimates (up to ~14% for women aged 20–59 in 2021–23 data)	(NHANES/CDC)	
Sub-Saharan Africa (WRA, pooled surveys)	≈30–45% (high, country-specific variation; many countries >40%)	(multi-country DHS analyses; regional surveys)	
Global (pregnant women)		(WHO fact sheet)	≈35.5% (WHO estimate, 2019–2023)
China (pregnant women/WRA)	Varies by study; often 20–30% in some hospital-based cohorts	(hospital/community studies)	

Notes: prevalence estimates are sensitive to definitions (overt vs. subclinical), assays (TSH cutoffs), sampling frames, and year. Country-level variations reflect true epidemiologic differences, diagnostic intensity, iodine status, and public-health program reach.

Table 2.
Anemia/iron deficiency prevalence (women 15–49) – selected countries/regions.

prevalence of ID in pregnancy is particularly concerning because pregnancy is a time of increased iron demand and because iron insufficiency during gestation is associated with maternal hypothyroxinemia in multiple studies [10, 11].

Global burden frameworks (GBD/WHO) and recent primary research emphasize major geographic inequalities and the role of social determinants (poverty, dietary patterns, infectious disease burden, access to supplementation and fortification programs) as the causes. From a policy standpoint, the persistence of high anemia prevalence, despite decades of supplementation programs, points to implementation gaps, multifactorial causation (dietary iron-bioavailability, infections, other micronutrient deficits), and the need for integrated strategies (fortification, improved antenatal care, infection control, and behavior change) [6, 10].

2.3 Overlap between iron deficiency and hypothyroidism

- The coexistence of ID and hypothyroidism is not coincidental. Both conditions disproportionately affect women, especially during pregnancy and postpartum, and are common in LMICs. Studies from India, Africa, and the Middle East consistently demonstrate a higher prevalence of IDA among patients with hypothyroidism compared to healthy controls [12, 13]. Furthermore, pregnant women with ID are more likely to exhibit impaired thyroid function, which can adversely affect fetal neurocognitive development [14].

- Thus, the dual burden of hypothyroidism and ID represents a **critical but often overlooked intersection** in endocrine and nutritional health. Moreover, symptoms of anemia and hypothyroidism overlap – fatigue, lethargy, cognitive slowing – making diagnosis and management challenging.

3. Physiology of thyroid hormone synthesis and the role of iron

The thyroid gland synthesizes two principal hormones – thyroxine (T4) and triiodothyronine (T3) – which regulate basal metabolic rate, growth, and development. The process of thyroid hormone biosynthesis is highly dependent on micronutrients.

3.1 Iodine uptake and organification

Iodine is transported into thyroid follicular cells via the sodium–iodide symporter (NIS). Inside the follicle, iodine is oxidized and incorporated into tyrosyl residues of thyroglobulin, a process termed organification.

3.2 Thyroid peroxidase (TPO): A heme-dependent enzyme

The oxidation and coupling reactions that generate T3 and T4 are catalyzed by **thyroid peroxidase (TPO)**, a heme-containing enzyme. Adequate iron is essential for heme biosynthesis and, consequently, for the activity of TPO. ID reduces TPO activity, leading to incomplete iodination of thyroglobulin and impaired thyroid hormone synthesis [15].

3.3 Peripheral conversion and micronutrient interactions

The conversion of T4 to T3 in peripheral tissues is mediated by selenium-dependent deiodinases, while zinc influences TSH synthesis and receptor function. ID indirectly affects these pathways by altering the cellular redox state and enzyme activity.

3.4 Role of iron beyond TPO

Iron also plays a role in oxygen transport through hemoglobin. Tissue hypoxia due to anemia may downregulate metabolic processes, mimicking hypothyroid symptoms. In addition, ID can impair neurotransmitter metabolism, potentially influencing hypothalamic regulation of TSH secretion.

In summary, **iron is indispensable for thyroid hormone biosynthesis and function**, and its deficiency sets off a cascade of biochemical impairments that culminates in hypothyroid manifestations**.**

4. Mechanisms linking iron deficiency to hypothyroidism

The association between ID and thyroid dysfunction is multifactorial. Several biochemical and physiological pathways are implicated:

4.1 Impaired thyroid peroxidase (TPO) activity

TPO, the enzyme responsible for iodide oxidation and coupling of iodotyrosine residues, is a **heme-containing enzyme**. ID reduces heme availability, directly impairing TPO activity [15]. This results in decreased formation of T3 and T4, even in iodine-sufficient people.

4.1.1 Thyroid peroxidase (TPO) gene and protein

The **TPO gene**, located on chromosome 2p25, encodes **TPO**, a key enzyme in thyroid hormone synthesis. TPO mRNA has 17 exons, producing the 933–amino acid TPO-1 protein. Alternative splicing yields shorter transcripts. Mutations of the TPO gene cause congenital hypothyroidism due to dyshormonogenesis.

TPO-1 is a dimeric glycoprotein with:

- **Ectodomain** (projects into follicular lumen; homologous to MPO, CCP, and EGF-like domains)
- **Short transmembrane domain**
- **Intracellular C-terminal region**

Though the full structure remains unresolved, molecular modeling has mapped active sites and predicted autoantibody epitopes. TPO, together with DUOX and caveolin-1, forms the **thyroxisome**, a complex essential for hormone synthesis at the apical thyrocyte membrane.

4.1.2 Epitope organization and autoantibodies

TPO is a major **autoantigen in autoimmune thyroid disease (AITD)**. Autoantibodies (TPOAbs) target two immunodominant regions:

- **IDR-A** and **IDR-B**, mainly within the MPO-like and CCP-like domains.
- Identified contact residues include amino acids 225, 353–386, and 597–775.

Nearly **all Hashimoto's thyroiditis (HT) patients** and **~75% of Graves' disease (GD) patients** have TPOAbs. They are also detectable in healthy individuals, particularly women, and predict future thyroid dysfunction. Longitudinal population studies confirm that rising TPOAbs correlate with an increasing risk of hypothyroidism.

In HT and GD, the distribution of IDR-specific antibodies is stable, heritable, and not disease-specific. Interestingly, some bispecific antibodies recognizing both **TPO and thyroglobulin** may be protective in HT [16, 17]

4.1.3 Clinical and oncologic relevance

- **Autoimmune thyroid disease**: TPOAbs and TPO-specific T cells mark thyroid infiltration and destruction. Genetic predisposition strongly links TPOAbs to AITD development.
- **Cancer**: TPO expression is reduced in thyroid and breast cancers (e.g., suppressed by the **BRAF V600E mutation** in thyroid cancer), while antigenicity persists. TPOAbs may even confer **protective effects against breast cancer** [16, 17]

4.1.4 Method of estimation of TPO enzyme activity

Quantitative and qualitative analysis of the TPO enzyme is done by the luminometric method, as shown in **Figure 1**. In brief, serum containing 0.1 to 0.2 mg/mL total protein will be incubated with 1.0 M glycine-NaOH (pH9.0) and 1.0 mM EDTA for 30 minutes with gentle shaking at 37°C, after which the reaction is initiated by the addition of 20 μL of luminol mix containing 1 M glycine-NaOH (pH 9.0), 1 mM EDTA, and 400 μM luminol. Following a 4.0-second delay, 5.0 μL of 80 mM H2O2 will be added, and the luminescence measured as relative luminescence units (RLUs) integrated over 10.0 seconds using a PerkinElmer Enspire Multimode Plate Reader [4].

One of the proposed mechanisms linking ID to hypothyroidism is impaired TPO activity, since TPO is a heme-dependent enzyme. Earlier animal studies by Zimmermann et al. and Beard et al. demonstrated that IDA significantly decreases TPO activity in rats [12, 15].

In contrast, a more recent human study by Savitha et al. examined both qualitative and quantitative aspects of TPO activity in 491 pregnant women. Participants were categorized into four groups: (i) iron-deficient hypothyroid, (ii) iron-deficient euthyroid, (iii) normal-iron hypothyroid, and (iv) normal-iron euthyroid. The proportion of detectable TPO activity was high across all groups – 98% in iron-deficient hypothyroid, 96% in iron-deficient euthyroid, 94% in

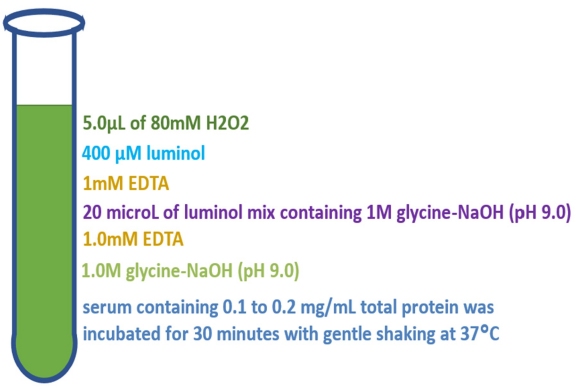


Figure 1.
TPO enzyme assay by luminometric method.

normal-iron euthyroid, and 87% in normal-iron hypothyroid women – with no statistically significant differences between them.

These findings are noteworthy because, contrary to expectations, TPO activity was not lower in the iron-deficient hypothyroid group compared with normal-iron euthyroid women. Several explanations may account for this divergence from earlier animal data: relatively modest sample sizes within subgroups, methodological differences in TPO activity measurement (chemiluminescence vs. fluorimetric assays), predominance of subclinical rather than overt hypothyroidism in the cohort, and potential preanalytical issues such as sample handling, processing, and storage of samples, since exposure to light can alter ferritin measurements [18].

4.2 Altered peripheral conversion of thyroid hormones

The conversion of T4 to the biologically active T3 is catalyzed by **selenium-dependent deiodinases**. Experimental studies suggest that ID disrupts cellular redox balance and impairs deiodinase activity, thereby reducing T3 generation [19].

4.3 Dysregulation of the hypothalamic–pituitary–thyroid (HPT) axis

ID has been shown to alter dopaminergic and noradrenergic neurotransmission, both of which modulate thyrotropin-releasing hormone (TRH) and TSH secretion [20]. This may lead to abnormal central regulation of thyroid function.

4.4 Reduced oxygen transport and tissue hypoxia

Anemia lowers oxygen delivery to tissues, producing **metabolic downregulation** that mimics hypothyroid physiology. In turn, hypothyroidism itself may blunt

erythropoietin production, creating a bidirectional relationship between anemia and thyroid dysfunction [21].

4.5 Immune modulation

Iron plays a role in lymphocyte proliferation and cytokine signaling. ID may alter immune function, potentially aggravating autoimmune thyroiditis in genetically predisposed individuals [22].

5. Clinical evidence

5.1 Observational studies

Several cross-sectional and case–control studies have demonstrated a strong association between IDA and hypothyroidism. Controlled experiments in animals show that ID lowers hepatic and thyroid heme pools and reduces TPO activity, producing measurable reductions in thyroid hormone synthesis and altered hormone kinetics. Seminal animal work by Hess and colleagues demonstrated decreased TPO activity in iron-deficient rats, with corresponding decreases in plasma thyroid hormone concentrations; Beard and coworkers similarly reported altered T3/T4 kinetics in iron-deficient rodents. These preclinical data provide a strong biochemical rationale for expecting clinically meaningful effects of iron status on the thyroid axis [12, 23].

Human observational data are consistent with the animal literature but are heterogeneous in magnitude and quality. Several cross-sectional and cohort studies report associations between low ferritin (or indices of ID) and higher TSH, lower free T4 or T3, or greater odds of subclinical hypothyroidism – particularly in pregnant women and adolescent girls, where iron demand is high. A 2023 systematic review and meta-analysis concluded that ID is associated with modest reductions in thyroid hormone concentrations and increased odds of thyroid dysfunction across studies, but highlighted important between-study heterogeneity and the predominance of cross-sectional designs that limit causal inference [24]. Recent pregnancy cohorts (including the Mysuru cohort and other hospital-based series) report associations between low ferritin and hypothyroxinemia in first-trimester women, a finding with potential implications for fetal neurodevelopment [25].

Eftekhari et al. reported that Iranian adolescent girls with low serum ferritin had significantly higher TSH and lower T4 levels than their iron-sufficient peers [13]. In a study done by Savitha et al., linear regression analysis showed an association between ferritin and T4 levels, with a significance of 0.003; hence, IDA was associated with hypothyroxinemia in pregnant women [25]. Similarly, data from Indian cohorts highlight high rates of concurrent ID in hypothyroid patients, especially in women of reproductive age [26].

5.2 Interventional studies

Clinical trials provide further evidence of causality.

In humans, Zimmermann et al. found that iron supplementation in iron-deficient, goitrous children improved thyroid hormone status, particularly when combined with iodine [27].

Trials in goitrous, iron-deficient children showed that **iron supplementation improves the response to iodine or iodized oil**, suggesting that correcting iron status restores TPO-dependent hormonogenesis and goiter regression in ID contexts. These trials – while relatively small and performed in settings with endemic goiter – argue that iron repletion can enhance thyroid recovery when iodination substrate (iodine) is available [27]. Beard et al. demonstrated in animal models that ID directly reduces thyroid hormone production, independent of iodine intake [12]. Animal supplementation studies likewise demonstrate partial restoration of TPO activity and thyroid hormone kinetics after iron repletion [19]. Taken together, these intervention data support a model in which ID is a reversible contributor to diminished thyroidal hormone output rather than an immutable cause of primary thyroid failure.

There are important clinical caveats. First, many human studies identify associations that are modest in absolute magnitude and concentrated among **subclinical** abnormalities rather than frank overt hypothyroidism. In clinical practice, therefore, ID may contribute to mild thyroid hypofunction or make existing borderline cases more likely to progress; it is less clearly established as a common independent cause of severe hypothyroidism in iodine-replete populations. Second, the interaction between **oral iron supplementation** and levothyroxine absorption is clinically relevant: simultaneous ingestion of ferrous sulfate can reduce thyroxine efficacy [28], sometimes producing biochemical hypothyroidism that resolves when dosing is separated. This pharmacokinetic interaction mandates pragmatic counseling (spacing iron and levothyroxine by several hours) and monitoring of TSH after initiating iron therapy in patients on thyroxine.

5.3 Pregnancy and maternal–fetal health

Pregnancy represents a critical window where both thyroid function and iron sufficiency are vital. ID during gestation is associated with **maternal hypothyroxinemia**, impaired thyroid hormone synthesis, and adverse fetal neurodevelopmental outcomes [14]. Moreover, ID may reduce the efficacy of levothyroxine therapy in pregnant women with hypothyroidism [29].

However, large randomized trials explicitly designed to test whether antenatal iron repletion prevents maternal hypothyroxinemia and improves neurodevelopmental outcomes in the offspring are lacking.

6. Clinical implications

6.1 Screening and diagnosis

Given the overlap in symptomatology – fatigue, cognitive impairment, cold intolerance – IDA and hypothyroidism often coexist unnoticed. Clinicians should consider **routine ferritin measurements** in patients with hypothyroidism, particularly women, adolescents, and pregnant patients.

6.2 Therapeutic considerations

- **Levothyroxine absorption**: oral iron supplements interfere with levothyroxine absorption when coadministered, necessitating careful timing of therapy [28].

- **Combined deficiency correction**: correction of ID enhances thyroid hormone synthesis and improves responsiveness to levothyroxine, highlighting the need for a combined nutritional–endocrine approach.

- **Pregnancy and lactation**: iron supplementation in pregnant women not only corrects anemia but also optimizes thyroid hormone availability to the fetus.

6.3 Public health perspective

In countries like India, where both IDA and hypothyroidism are highly prevalent, integrating **micronutrient supplementation programs** (iron, iodine, selenium) into maternal and child health initiatives could substantially reduce morbidity. Only iron supplementation can normalize thyroid hormones without thyroxine supplementation in antibody-negative, iron-deficient hypothyroid subjects.

7. Future directions and research gaps

Despite growing evidence, several areas require further exploration:

1. **Longitudinal studies**: large-scale prospective cohorts are needed to establish causality and clarify temporal relationships between ID and thyroid dysfunction.

2. **Intervention trials**: randomized trials comparing iron supplementation alone vs. combined with iodine or selenium in hypothyroid populations could provide actionable insights.

3. **Nutrigenomics and genetic susceptibility**: research into genetic polymorphisms influencing iron metabolism and thyroid autoimmunity may uncover high-risk subgroups.

4. **Public health strategies**: evaluating the cost-effectiveness of combined micronutrient fortification programs for the dual prevention of anemia and hypothyroidism.

8. Conclusion

ID and hypothyroidism are two highly prevalent conditions that often coexist, particularly in vulnerable populations such as women, adolescents, and pregnant women. Iron is indispensable for thyroid hormone biosynthesis through its role as a cofactor for TPO and its influence on peripheral conversion and central regulation. Clinical and experimental studies confirm that ID

adversely affects thyroid function and may worsen the course of hypothyroidism.

Early recognition and treatment of ID in hypothyroid patients can improve metabolic outcomes and optimize responsiveness to levothyroxine therapy. Integrating iron status assessment into thyroid disease management represents an essential yet underutilized strategy in clinical practice, especially in resource-limited settings where the dual burden of IDA and thyroid dysfunction is common.

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Chapter 5

Iron Metabolism and Hemochromatosis: Pathogenic Pathways to Multiple Organ Dysfunction – Clinical and Therapeutic Implications

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Abstract

Hemochromatosis is a systemic disorder of iron overload, marked by the gradual accumulation of iron in parenchymal tissues, which eventually results in dysfunction across multiple organs. It may be classified as primary, due to inherited genetic defects, or secondary, resulting from chronic transfusion therapy or disorders associated with ineffective erythropoiesis. Hereditary hemochromatosis is subclassified into four types. Types 1–3 are attributed to mutations in the HFE (High iron (Fe)), HJV (hemojuvelin), and TFR2 (transferrin receptor 2) genes, respectively, and exhibit autosomal recessive inheritance. In contrast, type 4, which is referred to as ferroportin disease, exhibits an autosomal dominant pattern of inheritance. The clinical spectrum encompasses hepatic injury (ranging from steatosis to cirrhosis and hepatocellular carcinoma), cardiomyopathy, pancreatic β-cell dysfunction leading to diabetes mellitus, arthropathy, endocrinopathies (such as hypogonadism), and cutaneous hyperpigmentation. Timely diagnosis – through assessment of serum iron indices (e.g. transferrin saturation and serum ferritin), molecular genetic testing, and liver biopsy when indicated – is critical to prevent irreversible organ damage. Management strategies primarily include therapeutic phlebotomy and/or iron chelation, aiming to maintain iron homeostasis. Emerging therapies, including hepcidin agonists and modulators of iron metabolism, represent promising avenues for future targeted treatment.

Keywords: iron overload, hemochromatosis, gene mutations, organ dysfunction, hepcidin therapy

1. Introduction

Hemochromatosis is a condition characterized by the buildup of iron in the parenchymal cells of various organs [1]. It can be caused by genetic mutations, or it can result from other conditions. Hereditary hemochromatosis is a genetic condition that is primarily found in individuals of Northern European ancestry [2]. This disorder occurs due to mutations in any gene that plays a role in controlling the absorption of iron into the bloodstream. The manifestations of hereditary hemochromatosis are contingent on the specific genetic mutations that are present. Type 1 is the most prevalent form of this condition, while types 2, 3, and 4 are considered rarer variants. Secondary hemochromatosis may occur as a consequence of frequent blood transfusions or pathologies that are associated with underlying disorders of erythropoiesis. The symptoms and signs of hemochromatosis usually manifest during adulthood and may include fatigue, joint pain, hyperpigmentation of the skin (also known as "bronze diabetes"), palpitations or shortness of breath due to heart failure, jaundice, ascites, or hepatosplenomegaly caused by liver damage, complications of diabetes mellitus due to damage to pancreatic beta-cells, gynecomastia, decreased libido, and body hair in patients with hypogonadism. The assessment of this condition mainly relies on serum ferritin concentrations, transferrin saturation (TSAT) levels, and genetic testing [3]. The treatment of hemochromatosis principally consists of regular phlebotomy, the purpose of which is to remove excess iron from the body, or iron chelation therapy. Early detection is important in order to prevent severe organ damage [4–6].

2. Iron metabolism

Heme iron from the diet is absorbed more quickly than non-heme iron. Iron absorption mainly takes place in the first parts of the small intestine (specifically the duodenum and the proximal jejunum) via a transmembrane protein known as HCP1 (Heme Carrier Protein 1) [3]. This protein is subject to upregulation in conditions of iron deficiency or hypoxia. A ferric reductase is the enzyme that plays a crucial role in converting ferric iron to its more absorbable ferrous form, thereby enhancing iron uptake in the body [7]. Divalent metal cation transporter 1 (DMT1) transports iron across the apical membrane into the enterocyte, where it can be stored as ferritin or released into circulation [5]. The mechanism by which iron passes through the basolateral membrane involves a protein called ferroportin (FPN), which requires another accessory protein known as hephaestin. FPN exports this ion from enterocytes, macrophages, hepatocytes, and the placenta, providing iron to the body for use by other tissues and cells [8, 9]. In addition to all this, hepcidin is a key molecule in regulating iron metabolism, being synthesized by the liver. Hepcidin acts by regulating the activity of FPN, binding to it and subsequently causing the protein to be internalized and degraded. This phenomenon decreases the release of iron from cells into the bloodstream, thereby affecting the overall levels of iron in the body [10]. Therefore, elevated hepcidin levels (which can occur during inflammation) will lead to downregulation of FPN, limiting iron absorption and its release from cells, while low hepcidin levels (in anemia, iron deficiency, or hypoxia) will stimulate iron absorption. In patients with hereditary hemochromatosis, mutations in the genes HFE, HJV, HAMP, and TfR2 disrupt hepcidin synthesis [11–13].

3. Etiology and pathogenesis

Hemochromatosis can be either primary (hereditary) or secondary (acquired). Hereditary hemochromatosis is a condition caused by genetic mutations that lead to excessive iron absorption. Four distinct forms of hereditary hemochromatosis have been described until now, according to the genetic defect involved. The first three types are all inherited in an autosomal recessive pattern. Type 4 hemochromatosis is unique because it follows an autosomal dominant pattern [3]. **Type 1** is the most prevalent type, caused by HFE gene mutations [14]. The C282Y variant has been identified as the most prevalent mutation. **Type 2** is linked to HJV gene mutations (also known as the HFE2 gene), which encode hemojuvelin (subtype 2a), or with mutations of the hepcidin antimicrobial peptide gene (HAMP) in subtype 2b [14]. Type 2 is also referred to as "juvenile" hemochromatosis due to its tendency to manifest at an earlier age, typically between 15 and 20 years of age. The mutated TFR2 protein is associated with **type 3** hemochromatosis. The symptoms of this condition may resemble those of type 1; however, it is possible that its onset may occur at an earlier age. **Type 4** is distinguished by its autosomal dominant pattern of inheritance, a departure from the autosomal recessive patterns observed in other types. The etiology of the condition is attributable to mutations in the SLC40A1 gene, which encodes the FPN transporter [4–6, 11, 15, 16].

Secondary iron overload is associated with hereditary or acquired anemia syndromes that require long-term transfusions, such as hemoglobinopathies (thalassemia and sickle cell disease are the most common), sideroblastic anemias, aplastic anemia, Blackfan-Diamond anemia, myelodysplastic syndromes, or osteomyelofibrosis. Secondary iron overload can also occur in chronic liver diseases (such as chronic hepatitis C or alcoholic hepatic steatosis), Friedreich's ataxia, porphyria cutanea tarda, intravenous iron therapy in end-stage renal disease, excessive dietary intake, and some rare conditions that affect iron metabolism, such as congenital atransferrinemia [2, 4, 12, 15].

Hepcidin deficiency has been identified as the primary cause of iron overload in hereditary hemochromatosis. Hepcidin is the primary regulator of iron homeostasis, controlling the amount of iron in the bloodstream. This protein achieves this by binding to FPN, the sole iron exporter protein found on the surface of duodenal enterocytes and macrophages. This interaction causes FPN to be internalized and degraded, which blocks iron export from these cells, thereby decreasing systemic iron levels [17]. It is imperative to note that HAMP, HFE, HJV, and TfR2 proteins are essential for regulating hepcidin expression in response to iron levels, as their absence leads to unregulated iron absorption and transport into the plasma, resulting in iron overload [11]. A proportion of this iron is absorbed by the bone marrow for the purpose of erythropoiesis. Hepatocytes store a certain amount of iron as ferritin, while skeletal muscles take up iron for incorporation into the structure of myoglobin. The human body lacks an efficient, active mechanism to excrete excess iron, making menstruation a crucial way for women to lose iron and maintain balance [11]. Excess iron released into the bloodstream from the liver can then enter other tissues, and it can cause damage to cellular structures. The circulating iron load depends on hepcidin, the master regulator of iron, and it is influenced by the HFE gene through its interaction with transferrin receptor 1 (TfR1) and transferrin receptor 2 (TfR2). HFE's role in sensing iron levels, especially when bound to TfR2, helps to regulate hepcidin production, thus controlling iron absorption and release into the circulation [18]. However, studies suggest that

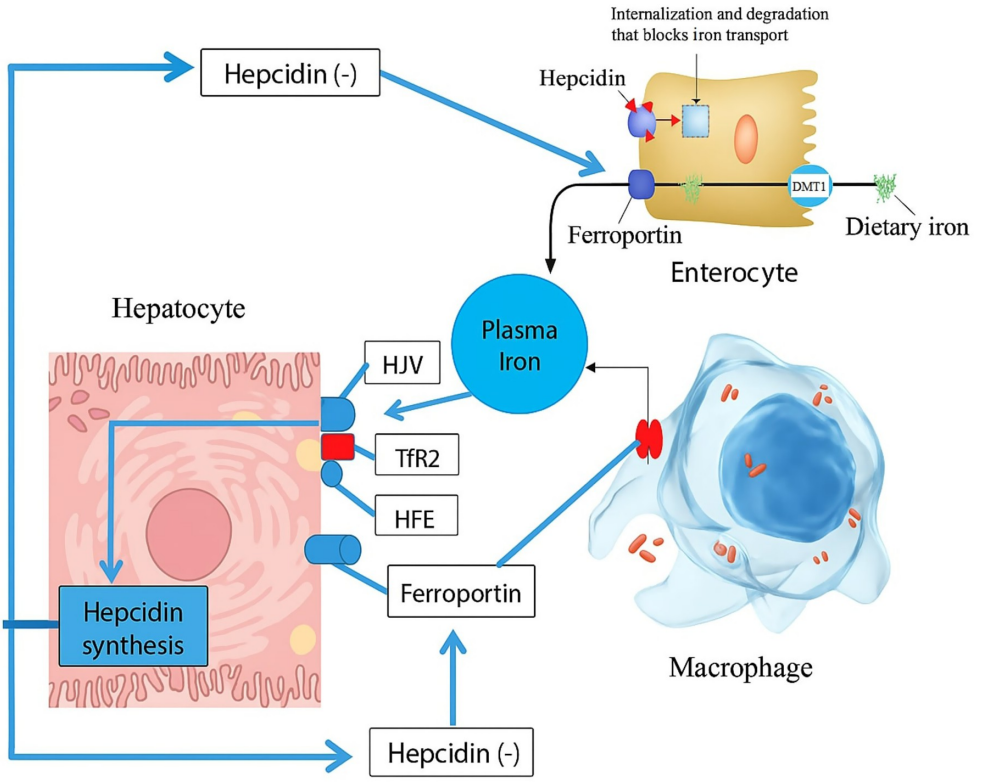


Figure 1.
Hepcidin deficiency is the main cause of hemochromatosis, a condition of iron overload where the body absorbs too much iron, leading to organ damage. In healthy individuals, hepcidin, a liver-produced hormone, controls iron release by binding to ferroportin (FPN) in cells, but in hemochromatosis, mutations in genes like HFE, TfR2, and HJV disrupt hepcidin production, causing excessive iron to enter the bloodstream [17]. Alternatively, rarely, ferroportin itself can be mutated, rendering it unable to respond to hepcidin [11].

HFE's influence on hepcidin may be dependent on HJV, with HFE acting in an HJV-dependent manner. While both HFE and HJV contribute to hepcidin regulation, HJV appears to be the primary regulator, with mutations leading to more severe consequences in iron homeostasis. Type 4 hemochromatosis is caused by mutations in the SLC40A1 gene, which encodes the FPN protein (see **Figure 1**). These mutations lead to FPN becoming resistant to hepcidin, the hormone that normally signals FPN to be degraded [11]. Hepcidin synthesis and release are appropriately induced by elevated plasma iron levels; however, the mutant FPN continues to release dietary iron into the plasma [5, 12, 16, 19].

4. The evolutionary adaptation of the C282Y mutation

The C282Y mutations are the main cause of hereditary hemochromatosis. Several studies have indicated that the C282Y mutation, which is believed to have originated approximately 6,000 years ago, aligns with the migration of Neolithic farmers into Central Europe and marks the beginning of the agricultural revolution [20].

The proliferation of this allele may signify an evolutionary adaptation to the significant dietary shift from iron-rich foods obtained through hunting to the consumption of iron-poor domesticated cereals and dairy products [20]. This transition in diet likely necessitated genetic changes to better utilize available iron sources, highlighting the interplay between human genetics and agricultural practices [20]. Although the European Mesolithic foragers depend on wild plants and animals for sustenance, which offered a considerable amount of iron, the Neolithic era initiated the domestication of plants and animals, resulting in a diet primarily based on dairy products and cereals. Milk products served as an effective alternative source of protein, while cereals provided an important source of calories. Thus, Neolithic people gradually adopted a diet very rich in carbohydrates, but poor in iron. Therefore, this type of diet based predominantly on cereals and dairy products had a number of consequences on iron metabolism [20]. First, phytates in cereals bind to iron, forming insoluble complexes that inhibit its absorption. Second, unlike human breast milk, which contains lactoferrin, animal milk has a very low iron availability. In addition, bovine milk can cause allergic-type reactions to the proteins it contains, causing chronic blood loss and secondary iron deficiency. Furthermore, Neolithic European farmers became more sedentary than Mesolithic European gatherers. Thus, the transition from the Mesolithic hunter–gatherer to Neolithic sedentary man and the availability of high-calorie foods led to an increase in the birth rate [20]. Given that pregnancy requires higher amounts of iron, with shorter interbirth intervals, a female may have few opportunities to restore her iron levels prior to subsequent pregnancies [20]. All these consequences of lifestyle changes exposed populations formed by Neolithic European farmers to the risk of acquiring iron deficiency throughout their lives (see **Figure 2**) [20].

All of these aspects constituted environmental selective pressures that encouraged the proliferation of mutations targeting genes involved in the pathogenesis of hereditary hemochromatosis. Therefore, variants of the HFE gene may have had a major benefit in avoiding iron deficiency during the transition to an agrarian lifestyle in Europe during the Neolithic era, but today, these mutations may be detrimental to modern humans [20, 21].

5. Epidemiology

The prevalence of hereditary hemochromatosis varies significantly by ethnicity, estimated at 1 in 200 to 500 people [23]. It is most frequently found in Northern Europeans, especially in those of Celtic and Scandinavian heritage, and is less common in African and Asian communities [5, 24]. Both men and women are equally affected by the condition, although the onset of hereditary hemochromatosis in women begins later (after age 50 in women; after age 40 in men) due to menstrual blood loss, which increases iron removal [25]. Men also have a higher prevalence of serious complications, such as cirrhosis and diabetes mellitus. The C282Y homozygous genotype prevalence is around 0.4%. This genetic variant is most commonly observed in Ireland, where its prevalence is notably higher, affecting about 1 in every 83 individuals. Hereditary hemochromatosis has a low clinical penetrance, despite a high prevalence of genetic mutations, with up to a quarter of patients with hereditary hemochromatosis being asymptomatic [4, 5, 12, 26].

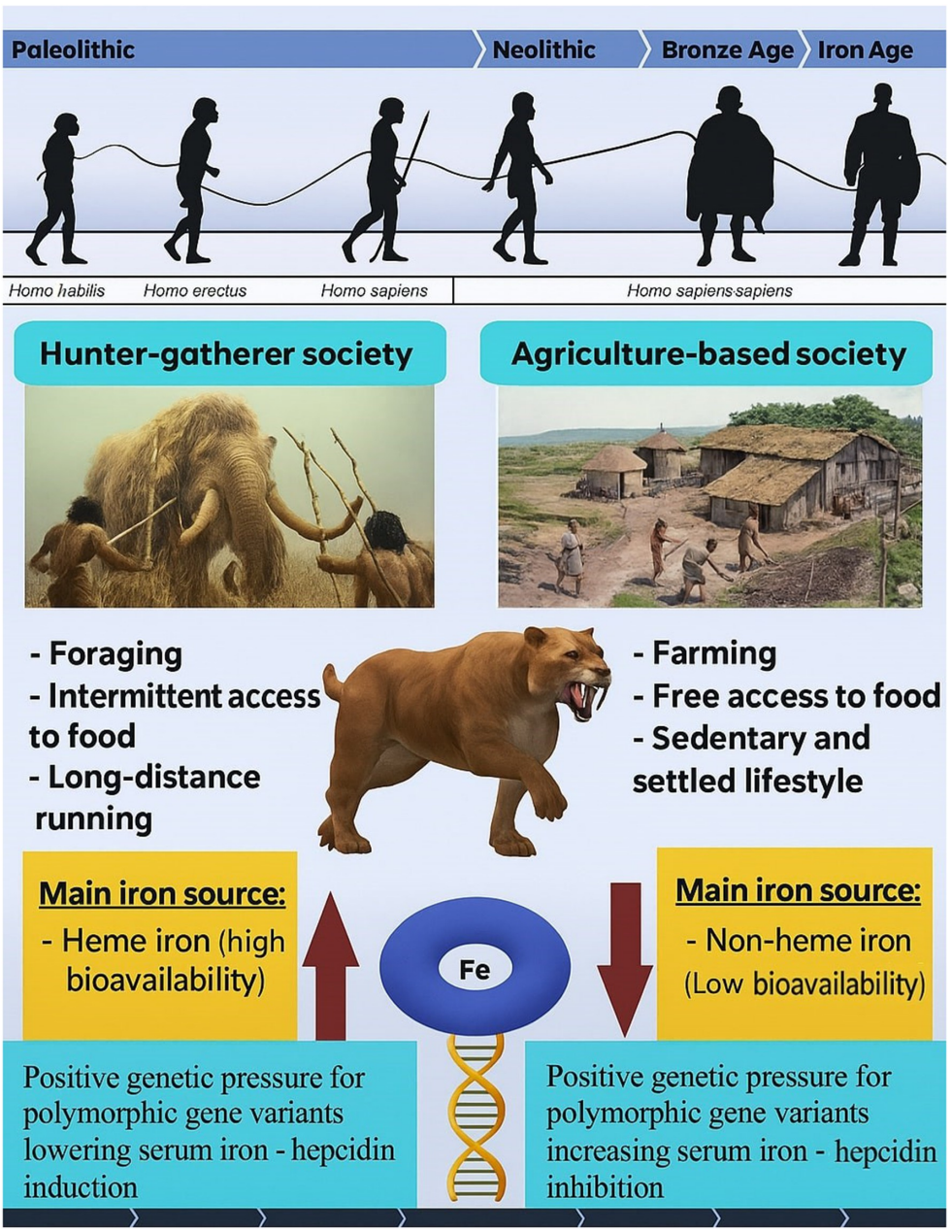


Figure 2.
Pathogens and hunger determined the priorities of human life and have shifted the trend of genetic selection throughout the millennia. The transition from a hunter–gatherer society to one based upon settled agrarianism, favored the development of opposite genetic traits of iron metabolism, greatly influencing human nutrition and disease [22].

6. Clinical manifestations based on pathophysiological mechanisms

The clinical presentation of patients diagnosed with hemochromatosis is determined by the affected organs and the severity of the lesions. Generally, patients remain asymptomatic until adulthood. It is noteworthy that patients typically remain

asymptomatic for a period of up to 10 years prior to receiving a diagnosis. In female patients, the onset of symptoms is typically later in the course of illness than in males. This is due to the loss of blood and the excretion of iron during menstruation [27].

6.1 Skin manifestations

Hemochromatosis can cause several distinctive skin changes. The most noticeable is hyperpigmentation, affecting over 90% of patients, where the skin appears darker, often described as a bronze or slate-gray color. This is frequently most prominent on sun-exposed areas and the face [28, 29]. Other skin features include thinning, dryness, and changes like ichthyosis. Additionally, hair loss, particularly in the pubic region, and koilonychia (spoon-shaped nails, often affecting the thumb, index, and middle fingers) can occur. The mechanisms underlying the pathophysiology of dermatological manifestations in patients with hereditary hemochromatosis remain incompletely understood. Although it was initially assumed that skin hyperpigmentation results from excessive melanin accumulation (stimulated by iron overload) and iron deposits in the skin, there is currently controversy regarding the role of iron deposits in hyperpigmentation. This fact has been emphasized by studies according to which, in patients with vitiligo, iron accumulation in the skin fails to neutralize the melanogenic defect. Another important cutaneous manifestation in patients with hereditary hemochromatosis is pruritus [28, 29]. There are two hypotheses regarding the mechanism by which iron overload may cause this manifestation. The first hypothesis supports the stimulation of class C fibers by iron deposits in the skin, while the second theory proposes that skin mast cells secrete increased amounts of histamine. However, the first theory is generally accepted because, in many cases, pruritus is resistant to antihistamine drugs [28, 29]. In addition, other complications of hereditary hemochromatosis, such as liver damage accompanied by cholestasis or hypothyroidism, can cause pruritus in this category of patients. Porphyria cutanea tarda is a pathological condition characterized by a lack of the uroporphyrinogen decarboxylase (UROD), resulting in the buildup of porphyrins, which are then deposited in the skin, causing photosensitivity and blistering [28, 29]. This deficiency can be either hereditary, due to genetic mutations, or acquired through various factors. Common triggers and contributing factors include alcohol consumption, estrogen therapy, viral infections (such as hepatitis C and HIV), iron overload, and smoking. In patients with hereditary hemochromatosis, iron overload can inhibit UROD, making people with hemochromatosis more susceptible to developing porphyria cutanea tarda or worsening a pre-existing condition [28, 29].

6.2 Osteoporosis and arthropathy in hemochromatosis

Osteoporosis is a recognized complication of hemochromatosis and can occur more frequently than expected in individuals with this condition. Bone loss results from increased bone resorption and decreased osteogenesis due to iron overload [30]. First, growing evidence indicates that iron promotes the differentiation and activation of osteoclasts. These require a significant amount of energy, which is supplied by numerous mitochondria. Mitochondrial biogenesis relies on iron, a crucial element for the proteins involved in the respiratory chain. The differentiation of osteoclasts was associated with increased expression of transferrin

receptor 1 (Tfr1) and improved iron absorption, which enhanced their ability to resorb bone. Conversely, the use of iron chelators diminished the bone resorption activity of osteoclasts [10]. This discovery was corroborated by demonstrating that ferric ions stimulated RANKL-induced osteoclast development in macrophages derived from bone marrow. Iron facilitates the generation of reactive oxygen species (ROS), which can promote osteoclastogenesis [10]. In addition to impacting osteoclastogenesis, increasing evidence indicates that iron also affects the activity of mature osteoclasts and bone resorption. Osteoclasts are characterized by their prominent expression of tartrate-resistant acid phosphatase (TRAP) [10]. Initial research demonstrated that blocking TRAP activity in osteoclasts eliminates bone resorption. It was proven that the mRNA of TRAP includes an iron regulatory element located in the 5′ -flanking region. This finding implies that the expression of TRAP is controlled by iron at the gene transcription level [10]. All these pathways may play a role in disrupted bone remodeling in conditions of iron excess. On the other contrary, iron overload can disrupt the delicate balance of bone remodeling by decreasing bone formation [10]. Thus, iron interferes with the Wnt/β-catenin pathway, disrupts the BMP-2/SMADs pathway, or downregulates the PI3K/AKT/mTOR pathway, all of these signaling pathways being crucial for osteoblast differentiation and bone formation. In addition, iron overload may indirectly affect bone through complications like liver cirrhosis or hypogonadism associated with hemochromatosis [10, 31, 32].

Arthropathy is a common and often early manifestation of hereditary hemochromatosis, in which iron buildup in joints results in progressive damage to these structures. Similar to osteoarthritis, arthropathy in hereditary hemochromatosis involves cartilage thinning, bone damage, and the formation of osteophytes. Although osteoarthritis typically affects weight-bearing joints, the second and third metacarpophalangeal joints are the most commonly affected in hereditary hemochromatosis, but involvement of wrists, knees, hips, and ankles is also possible [12, 31]. In addition, hereditary hemochromatosis arthropathy can occur at a younger age than typical osteoarthritis. Chronic, indolent pain and stiffness in the affected joints are common symptoms. The deposition of calcium pyrophosphate crystals in the joints, also known as pseudogout, is a hallmark of hereditary hemochromatosis arthropathy and can cause acute joint pain. Although primarily degenerative, hereditary hemochromatosis arthropathy can have an inflammatory component, with some features resembling rheumatoid arthritis. Venesection is the primary treatment for hereditary hemochromatosis, but it often provides limited relief for joint symptoms and does not stop the progression of joint damage [12, 31].

6.3 Liver involvement

Excessive iron deposition in the liver leads to the generation of ROS through the Fenton reaction, resulting in oxidative damage to intracellular organelle membranes and DNA strand breaks in hepatocytes. These processes subsequently cause cell death by apoptosis, necrosis, or ferroptosis [33].

Liver dysfunction is present in 75% of patients. Jaundice typically does not manifest in the initial phases of the disease. The clinical manifestations of liver disease can encompass a wide spectrum of presentations, including abdominal discomfort, hepatomegaly and splenomegaly, ascites, jaundice, and upper gastrointestinal bleeding, which can result from portal hypertension. Although

cirrhosis manifests in only 10% to 15% of patients, as many as 30% of individuals suffering from hemochromatosis and cirrhosis have been found to develop hepatocellular carcinoma, which is a significant cause of mortality in this category of patients [4, 12]. Advanced hepatic fibrosis or cirrhosis in hemochromatosis associated with HFE gene mutations is an uncommon occurrence in patients younger than 45 years without comorbidities, manifesting in only 8%–25% of all C282Y homozygous patients. Risk factors encompass excessive alcohol consumption, diabetes mellitus, serum ferritin levels greater than 1,000 μg/L, liver iron concentration greater than 200 μmol/g, and onset of joint damage [4, 12].

6.4 Endocrine dysfunction

Hemochromatosis, characterized by excessive iron accumulation, frequently leads to endocrine dysfunction, particularly diabetes mellitus and hypogonadotropic hypogonadism. These complications are often associated with advanced disease, including liver cirrhosis. The most common endocrinopathy in hereditary hemochromatosis is hypogonadotropic hypogonadism, which manifests as diminished libido (experienced by 26% of patients) and erectile dysfunction (45% of men) [34]. This impairment of the hypothalamic-pituitary axis causes insufficient gonadotropin secretion, while damage to the Leydig cells directly impairs testosterone production, creating a dual mechanism for hypogonadotropic hypogonadism [34]. Adrenal insufficiency can also occur in individuals with hemochromatosis. While not a common complication, it can arise due to iron deposition in the adrenal glands, disrupting their function, or it can result from iron-induced pituitary dysfunction, which reduces the ability to secrete tropic hormones. A lack of ACTH means the adrenal glands do not receive the signal to produce sufficient cortisol, resulting in adrenal insufficiency [34]. Hypothyroidism is another disorder that has been observed in individuals diagnosed with hereditary hemochromatosis. However, its prevalence is extremely low, which can be explained by the greater sensitivity of pituitary glandular cells to the damaging effects of iron deposits compared to thyroid cells [34].

Iron is critical for pancreatic beta-cell function, as it is involved in the synthesis and processing of insulin and in the production of ATP and ROS necessary for insulin secretion. Thus, it is a key component of proteins containing iron–sulfur clusters, which are essential for the function of various enzymes involved in insulin synthesis and processing within beta-cells [35]. Iron also plays an important role in the signaling pathways that lead to insulin secretion in response to elevated blood glucose levels. In addition, iron is involved in the electron transport chain within mitochondria, where it is essential for ATP production. Pancreatic beta-cells have a unique, increased iron metabolism due to their essential role in producing insulin, requiring higher levels of iron transport and storage proteins compared to alpha and delta-cells [35]. Excess iron leads to ROS accumulation, causing oxidative damage to pancreatic beta-cells. Pancreatic beta-cells are particularly vulnerable to this damage due to their high metabolic activity and ROS production, which overwhelm their limited antioxidant defenses. The β-cells in the pancreas exhibit lower levels of antioxidant enzymes like glutathione peroxidase (GPx), superoxide dismutase (SOD), and catalase compared to other cell types [35]. Consequently, maintaining an optimal level of iron is crucial for the proper functioning of beta-cells; however, an excess of iron can be harmful, mainly due to the production of ROS.

The phenomenon of iron-induced beta-cell toxicity is primarily linked to the buildup of ROS via a mechanism referred to as Fenton's reaction [35]. ROS excess has been demonstrated to cause DNA breakdown, lipid and protein oxidation, which in turn leads to mitochondrial damage and apoptosis. It has been demonstrated that ROS has the capacity to exert a direct influence on circulating human insulin through a process of hydroxylation of phenylalanine residues. This, in turn, results in a reduction in the affinity for binding to the insulin receptor. Hypoxia-inducible factors (HIF) are pivotal to the regulation of the cellular response to low oxygen levels [36]. This is achieved by the upregulation of specific proteins that play a role in the regulation of glucose metabolism, the development of new blood vessels, and erythropoiesis [5]. The regulation of HIF protein levels by cellular iron levels is achieved through the control of prolyl-hydroxylase (PHD) activity. PHDs require ferrous iron and molecular oxygen as cofactors to catalyze the hydroxylation of the HIF-alpha subunit. This hydroxylation creates binding sites on the HIF-alpha protein, marking it for recognition by the von Hippel-Lindau (VHL) E3 ubiquitin ligase and subsequent degradation by the proteasome [5]. Decreased HIF activity in beta-cells can lead to increased anaerobic glycolysis. In the absence of sufficient HIF, the beta-cell may overcompensate by increasing glycolytic flux, even when oxygen is available, potentially disrupting normal glucose-stimulated insulin secretion. Consequently, the reduced level of HIF-1α results in a reduction in glucose-stimulated ATP production and causes β-cell dysfunction [35, 37–40]. This constitutes an additional important mechanism through which iron overload can precipitate β-cell dysfunction and increase the incidence of diabetes. Furthermore, the potential of iron to induce ferroptosis, a non-apoptotic form of cell death characterized by the accumulation of iron ions, has been identified as a contributing factor to β-cell dysfunction and death [35, 37–40].

6.5 Risk for infections

Individuals with hemochromatosis are more susceptible to certain infections, particularly those caused by bacteria that thrive in iron-rich environments. Specifically, Vibrio vulnificus, Yersinia species, Listeria, and other bacteria can be more dangerous to individuals with hemochromatosis. This increased risk is a result of the additional iron, which promotes the proliferation and virulence of these microorganisms [41]. Many bacteria, fungi, and even some viruses require iron for growth and survival. In hemochromatosis, the body absorbs and stores excessive amounts of iron, creating an ideal environment for these pathogens to flourish. Vibrio vulnificus, a bacterium commonly found in raw seafood, is particularly dangerous for individuals with hemochromatosis. It can cause severe, life-threatening infections, including sepsis, in those with iron overload. Hemochromatosis can also affect the immune system, potentially impairing the body's ability to fight off infections effectively. For example, iron overload can increase the number and activity of suppressor T-cells while decreasing the activity of helper T-cells, thus altering the immune response [4].

6.6 Cardiac involvement

Iron overload cardiomyopathy is defined as systolic or diastolic cardiac dysfunction secondary to excessive iron deposition in the heart, and it can lead to

congestive heart failure in patients with hemochromatosis. In addition to its role as a significant cause of morbidity, iron overload cardiomyopathy has been identified as a major contributing factor to mortality, accounting for approximately one-third of hereditary hemochromatosis deaths, especially in young males [14]. Concurrently, cardiac involvement is the primary cause of mortality associated with thalassemia major. Iron is transported into myocytes through voltage-gated L-type calcium channels [14]. The effects of iron deposition on the conduction system are less pronounced compared to the active myocardium. The deposition of iron has been observed in the cardiac conduction system in general, and particularly in the atrioventricular node. The occurrence of complete atrioventricular block, a condition precipitated by iron deposition, may necessitate the insertion of a permanent pacemaker. Free iron contributes to the production of ROS via the Fenton reaction, resulting in the generation of hydroxyl radicals that are harmful to cardiomyocytes [14]. Simultaneously, the excitation–contraction coupling can be impaired by increased concentrations of iron that will be transported through L-type calcium channels and which affect calcium transport in cardiomyocytes [42]. This phenomenon is associated with the progression of both systolic and diastolic ventricular dysfunction [14]. In addition to direct myocardial damage, iron overload may also exert an indirect effect on the heart through its effects on other organ systems. Consequently, liver dysfunction, diabetes mellitus, and endocrinopathies may exacerbate heart failure in these patients. It is important to note that diastolic and systolic dysfunction of the left ventricle may be masked by an increased cardiac output state caused by anemia in patients with certain hematological disorders characterized by ineffective erythropoiesis or hemolytic processes [14, 43, 44].

7. Diagnostic tests

In cases of suspected hemochromatosis, the initial tests of serum ferritin and serum TSAT are crucial, as elevated levels may suggest possible iron overload. Further testing is recommended if serum ferritin exceeds 200 μg/L in menstruating women or 300 μg/L in men/non-menstruating women, or if TSAT goes above 40% (women) or 50% (men) [5]. A normal ferritin value combined with a TSAT of <45% has a negative predictive value of 97% for hemochromatosis [4, 5, 44].

In instances where a patient exhibits increased serum ferritin and TSAT levels above the values already mentioned, the next step to establish a definitive diagnosis is genetic testing. Up to 95% of all cases of hereditary hemochromatosis are caused by homozygous C282Y and compound heterozygous C282Y/H63D mutations of the HFE gene. Rarely, patients with hemochromatosis have non-HFE mutations. Mutations in the HJV (Juvenile Hemochromatosis), HAMP (Hepcidin), and TFR2 genes are associated with forms of hereditary hemochromatosis that typically present earlier and are more severe than the most common form, which is caused by HFE mutations [5]. Transaminases are typically elevated, though not generally to a level exceeding twice the standard value. Liver biopsy is the procedure used to measure hepatic iron content and assess the degree of liver damage. Histopathological analysis in hemochromatosis involves identifying iron deposition as golden-yellow hemosiderin granules within hepatocytes and other cells, especially with the use of a Prussian blue stain [5]. In instances where the diagnosis remains uncertain in patients with non-HFE mutations, a diagnostic liver biopsy may be indicated. While a liver biopsy can definitively diagnose hepatocellular carcinoma

and quantify fibrosis, it is not necessarily the ideal procedure due to its invasive nature and associated risks [5]. Magnetic resonance imaging (MRI) is a non-invasive and increasingly preferred method for quantifying liver iron deposition compared to the invasive liver biopsy, as it offers a safer and more convenient way to diagnose, grade, and monitor iron overload disorders. Fibrosis-4 Index (Fib-4), Hepascore, and transient elastography (FibroScan) are also non-invasive techniques used to detect liver fibrosis, including in patients with hereditary hemochromatosis. Transient elastography, known as FibroScan, is a non-invasive ultrasound-based method to measure liver stiffness and assess fibrosis. It is an outpatient procedure that generates immediate results in kilopascals (kPa) and is a reliable tool for identifying cirrhosis [5].

Hereditary hemochromatosis is linked with an increased risk of type 2 diabetes, a condition that arises from pancreatic iron deposition. The diagnosis of type 2 diabetes can be confirmed by analyzing fasting serum glucose levels, conducting glucose tolerance tests, and measuring HbA1c concentrations. It has been hypothesized that glycosylated hemoglobin levels may not be reliable in patients exhibiting high red cell turnover. MRI is a highly effective procedure for the assessment of iron deposition in the pancreas. In cases of coexisting infertility or reproductive system disorders, assessing serum FSH (follicle-stimulating hormone), LH (luteinizing hormone), and testosterone, along with GnRH (gonadotropin-releasing hormone), is crucial for diagnosing hypogonadism by differentiating between primary (testicular) and secondary (hypopituitary/hypothalamic) causes [5]. The use of brain MRI has been demonstrated to be a reliable method for the detection of pituitary iron deposits [5].

In advanced stages of hemochromatosis, electrocardiographic findings may include low-voltage QRS complexes and nonspecific T-wave or ST-segment abnormalities. Concurrently, the most prevalent atrial tachyarrhythmia identified in patients with hemochromatosis is paroxysmal atrial fibrillation. The process of iron deposition within the conduction system has been demonstrated to induce a range of cardiac complications, including atrioventricular block. Echocardiography is crucial for the early detection of left ventricular diastolic dysfunction secondary to restrictive cardiomyopathy, which gradually evolves into dilated cardiomyopathy with low ejection fraction [45]. Cardiac MRI is a quantitative assessment tool for myocardial iron load in patients with hemochromatosis. However, the use of this procedure in clinical practice is limited by its high cost and low availability [5, 44].

Joint pain is a common early symptom of hereditary hemochromatosis, with some studies indicating it may affect up to 75% of patients before a definitive diagnosis is established. Initially, standard radiography can help identify the arthritis caused by iron overload [5]. The presence of osteopenia and osteoporosis can be analyzed by means of a dual-energy X-ray absorptiometry (DEXA) scan, which is based on bone density measurement [5].

8. Management

Hemochromatosis treatment primarily focuses on reducing iron levels in the body to prevent complications. The particular treatment approach will be determined by the cause of hemochromatosis and the extent of iron overload. The primary therapeutic options include:

- Phlebotomy – this is the key therapeutic intervention in managing hereditary hemochromatosis. It involves regularly removing a certain amount of blood to decrease iron levels in the body. Initially, the goal is to perform this procedure once or twice a week, with each session typically removing a unit of blood (about 500 mL). The primary objective during this stage is to lower serum ferritin levels to below 50 μg/L, while it is preferable to keep TSAT under 50%. It is recommended to assess hemoglobin levels during each phlebotomy and to evaluate serum ferritin levels at every fourth procedure [6, 12, 26]. Subsequently, when the serum ferritin level drops below 200 μg/L, it is recommended to measure ferritin levels at each phlebotomy session. Once target iron levels are reached, the frequency of phlebotomy can be reduced to maintenance therapy, which may involve phlebotomy every two to four months, in order to ensure that serum ferritin levels remain within the optimal range of 50–100 μg/L [6, 12, 26]. In the maintenance phase, it is recommended that ferritin levels be monitored two to three times per year [6, 12, 26].

- Erythrocytapheresis – this procedure is an alternative to conventional phlebotomy, involving the selective removal of red blood cells, allowing for a more targeted reduction of iron levels than phlebotomy. In erythrocytapheresis, blood is collected from the patient, and red blood cells are separated from the plasma and other blood components [6, 12]. The red blood cells, which hold a significant amount of the excess iron, are extracted, but plasma proteins and platelets are reintroduced to the patient [6, 12]. Compared to standard phlebotomy, erythrocytapheresis can achieve a greater reduction in iron load per session. In addition, erythrocytapheresis requires fewer overall treatment sessions to obtain the same therapeutic effect and, thus, it may be more comfortable for patients with hemochromatosis. In clinical practice, erythrocytapheresis should be considered for individuals with heart failure, liver disease, in cases of hypoproteinemia, in thrombocytopenic patients, or in those who cannot tolerate hypovolemia induced by phlebotomy, because it can preserve blood volume [6, 12]. However, this procedure may have some disadvantages, such as higher cost and the need for specialized equipment and experts who know how to use it. In addition, erythrocytapheresis is also associated with other potential complications, such as hypocalcemia (due to the citrate anticoagulant used), risk of infections, and allergic reactions [6, 12].

- Chelation Therapy – For patients who cannot undergo phlebotomy (for instance, those with anemia or cardiovascular issues), iron-chelating agents such as deferoxamine, deferasirox, or deferiprone may be used. These medications help bind excess iron and promote its excretion from the body. Deferoxamine is a parenteral iron chelator used primarily for acute iron overload situations, particularly those arising from blood transfusions. Deferasirox proves effective for patients with transfusion-dependent anemia, and it is administered orally [6, 12]. Deferiprone is also an oral iron chelator, mainly used in patients with thalassemia and other types of anemia that require periodic blood transfusions. The effectiveness of these drugs in iron excretion is similar. Although iron-chelating agents are not as effective as phlebotomy in the management of hereditary hemochromatosis, they are more beneficial for patients with hemolytic anemia or disorders of erythropoiesis accompanied by iron overload [6, 12].

- Dietary Changes – Patients may be advised to avoid iron-rich foods (such as red meat) and foods or supplements containing vitamin C, which increase iron absorption. Patients with hemochromatosis should avoid alcohol consumption, as it can cause further liver damage and also enhance iron absorption by inhibiting the production of hepcidin [41]. Individuals with hereditary hemochromatosis should also avoid raw or undercooked seafood (especially clams and oysters) in order to minimize infection risk with Vibrio vulnificus, a bacterium that thrives in iron-rich environments and causes severe infections in individuals with hemochromatosis [6].

- Monitoring and Management of Complications – Regular monitoring of liver function and other potential complications (like diabetes mellitus, skin changes, or heart problems) is essential, as hemochromatosis can lead to serious health issues if left untreated. When hemochromatosis leads to significant liver damage, causing cirrhosis or liver failure, a liver transplant may be considered. Despite a relatively high prevalence in the European population, hereditary hemochromatosis is responsible for only about 1% of liver transplant cases [12]. This occurs because only a minor fraction of patients suffering from hereditary hemochromatosis accumulate such significant quantities of iron in the liver that they are likely to develop cirrhosis [46]. Another proven benefit of liver transplantation is the fact that it changes the underlying pathophysiological mechanism that occurs in hereditary hemochromatosis and thus establishes normal hepcidin levels [6].

In the case of patients who develop endocrine dysfunction, they will require the administration of hormone replacement therapy, while individuals with arthropathy will need physiotherapy, the administration of nonsteroidal anti-inflammatory drugs, analgesics, and even joint replacement. Patients who develop osteoporosis will need vitamin D and bisphosphonates [6].

- Hepcidin mimetics, such as Rusfertide, which have been successfully used in the treatment of polycythemia vera, hold significant promise for the future treatment of hemochromatosis. Rusfertide was recently used in a clinical trial that included patients with hereditary hemochromatosis type I during the maintenance phase. This treatment slows down the absorption of iron and accelerates its redistribution, proving useful in reducing the need for phlebotomy and lowering the level of TSAT. However, Rusfertide cannot cause iron loss, thus making it ineffective for the depletion phase. Currently, there is insufficient evidence to support the use of hepcidin-based therapy until further information from clinical trials becomes available [6, 12].

9. Prognosis

The prognosis for individuals diagnosed with hemochromatosis has progressively improved over recent decades due to advances in the early detection and treatment of the condition. The extent of liver damage serves as the main prognostic factor at the time of diagnosis for these individuals [4, 16]. The ferritin level at the time of diagnosis is also of prognostic significance. Patients exhibiting ferritin levels in excess of 2000 μg/L have been observed to demonstrate higher mortality rates in

comparison to those exhibiting levels of 1000 μg/L or less. Thus, early diagnosis of patients with hemochromatosis and regular treatment with phlebotomy, accompanied by iron chelation therapy, can prevent complications that may occur in the evolution of the disease and can increase survival in patients in whom complications are already established. Patients who develop severe liver failure may be eligible for a liver transplant. This treatment has been shown to be efficacious in the management of the condition, as the transplanted liver will be capable of normal hepcidin production [4, 16].

10. Conclusion

In summary, hemochromatosis can be a genetic disorder, or it can be secondary to other conditions, consisting of abnormal absorption and excessive storage of iron in various organs and tissues. Iron overload can cause irreversible damage to organs, but early diagnosis can help to effectively manage this condition and avoid complications [35].

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Chapter 6

Managing Iron Deficiency Anemia in Advanced Chronic Kidney Disease and Dialysis

Karen Courville, Norman Bustamante and Jermaine Tomlinson

Abstract

Iron deficiency is a primary driver of anemia in patients with advanced chronic kidney disease (CKD) and those on dialysis, contributing to poor quality of life, cardiovascular risk, and increased mortality. The pathophysiology is complex, involving both absolute iron deficiency from blood loss and poor intake, and functional deficiency driven by elevated hepcidin, which blocks iron absorption and mobilization. Management strategies differ by clinical context. While oral iron is an option for nondialysis patients, its utility is often limited by poor absorption and gastrointestinal side effects. Intravenous (IV) iron is the standard of care for dialysis patients and those who fail oral therapy, offering effective repletion of iron stores. The landmark PIVOTAL trial has shifted practice by demonstrating that a proactive, high-dose IV iron regimen in hemodialysis patients significantly reduces mortality, cardiovascular events, and the need for erythropoiesis-stimulating agents compared to a reactive approach, without increasing infection rates. However, iron therapy must be individualized, particularly in special circumstances such as active sepsis, where iron administration is typically deferred to avoid worsening the infection. This review covers the principles of diagnosis and treatment of iron deficiency anemia in advanced CKD, comparing iron replacement strategies and addressing complex clinical scenarios.

Keywords: chronic kidney disease, iron deficiency anemia, intravenous iron, oral iron, dialysis, nondialysis

1. Introduction

Anemia is one of the most prevalent and clinically significant complications in patients with chronic kidney disease (CKD), especially in stages 4 and 5 and in those undergoing dialysis [1]. It is associated with diminished quality of life, reduced exercise tolerance, cognitive impairment, increased cardiovascular risk, hospitalizations, and mortality, as well as greater healthcare costs and sensitization risks that may complicate kidney transplantation eligibility [2]. Iron deficiency is a major contributor to anemia in CKD, coexisting with other mechanisms such as

erythropoietin (EPO) deficiency, shortened red blood cell (RBC) lifespan, chronic inflammation, and uremic toxins [3].

Iron deficiency can be absolute, characterized by depleted iron stores, or functional, where iron stores are preserved but unavailable for erythropoiesis due to increased hepcidin levels and iron sequestration [4]. Hepcidin, a hepatic hormone regulated by inflammation, iron status, hypoxia, and erythropoietic activity, plays a central role in the development of functional iron deficiency [5]. It inhibits iron absorption in the gut and iron release from macrophages by promoting the degradation of ferroportin, the only known iron exporter [6]. In CKD, elevated hepcidin levels due to inflammation and decreased clearance contribute to impaired iron availability and hyporesponsiveness to erythropoiesis-stimulating agents (ESAs) [7].

The management of iron deficiency anemia in CKD has evolved considerably in the past decade [8]. Oral iron remains an option for selected nondialysis patients, but gastrointestinal intolerance and poor absorption limit its utility [9]. Intravenous (IV) iron is the standard of care in dialysis patients, and new formulations with improved safety profiles, such as ferric carboxymaltose (FCM) and iron isomaltoside, have expanded treatment options [10]. However, concerns remain regarding potential adverse effects, including infections, oxidative stress, vascular calcification, and rare events such as hypophosphatemia [11].

Despite the availability of guidelines and therapies, anemia in CKD remains undertreated. Observational data show that a large proportion of patients do not receive adequate evaluation or treatment for anemia, and clinical inertia is especially marked regarding iron therapy [12, 13]. This chapter will review the principles of diagnosis and treatment of iron deficiency anemia in advanced CKD, compare oral versus IV iron strategies, and address special clinical scenarios, such as sepsis, infection, and the predialysis transition period, where iron therapy decisions are more complex and must be individualized.

2. Treatment of iron deficiency in adults

The choice between oral or IV iron depends on numerous factors, including the severity of anemia, cost, availability, as well as tolerance to oral preparations.

2.1 Oral iron therapy

Oral treatment is the first line in most patients because of its wide availability, low cost, safety, and effectiveness when taken appropriately. However, up to 70% of patients treated with oral iron experience gastrointestinal adverse effects. The use of oral iron supplements is indicated to treat iron deficiency anemia, manage iron deficiency without anemia, and provide nutritional support to prevent deficiency. Oral treatment may not be effective in patients with blood loss, inflammatory bowel disease, gastric bypass, or CKD [14, 15].

2.1.1 Choosing the oral formulation

The most appropriate form is liquid or tablets of ferrous salts. Adverse effects are similar in different presentations; however, alternate-day treatment schemes and liquid presentations may reduce side effects. There is no evidence that one

presentation is more effective or has fewer adverse effects than the other. The use of enteric-coated or sustained-release tablets is not recommended, as they are poorly absorbed due to the release of iron very distally in the intestine. Some available preparations include Ferric citrate (210 mg elemental iron), Ferric maltol (30 mg elemental iron), Ferrous fumarate (106 mg elemental iron), Ferrous gluconate (27–38 mg elemental iron), and Ferrous sulfate (65 mg elemental iron) [16].

2.1.2 Dosage and administration

The use of high doses may initially be counterproductive, as it decreases iron absorption and increases side effects, without improvement of iron stores or anemia. There are two dosing schedules with marginal differences in efficacy [17–20]:

- **Daily doses:** Preferable, as it is easy to remember and can theoretically increase iron absorption.

- **Alternate-day doses:** May be recommended to reduce gastrointestinal effects and may result in equivalent or better iron absorption compared to daily doses. High doses of iron per day can paradoxically decrease iron absorption due to increased hepcidin levels.

2.1.3 Duration of therapy and absorption

Treatment can be continued for up to six months after hemoglobin levels have normalized to ensure adequate iron stores have been achieved. Measures to improve oral absorption are uncertain, and their use should be discussed with patients. These include avoiding coadministration with foods containing phosphates, dairy, calcium supplements, coffee, tea, and eggs. As iron is best absorbed in an acidic medium, drugs that alter gastric acidity (e.g., antacids) can decrease absorption and should be given two hours before or four hours after the iron supplement. Coadministration of vitamin C can improve iron absorption, although its effect is usually minor [21].

2.1.4 Adverse effects

The main adverse events are gastrointestinal, including metallic taste, nausea, vomiting, flatulence, constipation, and epigastric pain. Black stools may also be observed. These effects are seen with all oral presentations, with reported rates for ferrous fumarate at 43%, ferrous gluconate at 31%, and ferrous sulfate at 30% [22].

2.2 IV iron therapy

IV iron may be preferred in cases of poor adherence or gastrointestinal side effects from oral therapy, when there is a need to quickly replenish iron stores, in the context of active bleeding that exceeds the ability to replenish stores orally, in conditions that interfere with oral absorption, or in the presence of an inflammatory state that interferes with iron homeostasis. The use of IV iron may reduce the need for blood transfusions [23, 24].

2.2.1 Choosing the IV iron formulation

The main formulations are FCM, ferric gluconate (FG), ferumoxytol, iron sucrose (IS), ferric derisomaltose, and low molecular weight iron dextran (LMW ID). All preparations are equally effective, with a similar safety profile. The main differences include the cost and the number of doses required. Regimens that use preparations that can be administered in a single dose are recommended to reduce the risk of complications from multiple infusions [25].

2.2.2 IV iron dosing and administration

The dose of IV iron is calculated based on body weight, current hemoglobin, and the amount of elemental iron in the chosen product, with the goal of treating anemia or replenishing stores. Fixed doses of about 1,000 mg are usually sufficient to treat anemia and replenish stores without causing overload. There is no evidence that doses greater than 1,000 mg of elemental iron are clinically superior [26].

2.2.3 Premedication and adverse effects

It is recommended not to premedicate patients without asthma or a history of allergic reactions to other medications. For patients with asthma or a history of drug allergy, methylprednisolone and H2 antihistamines may be used before IV iron infusion. The use of H1 antihistamines is not recommended for the prevention or treatment of infusion reactions, and premedication with diphenhydramine has been associated with an increase in such reactions. CM can cause hypophosphatemia and should be avoided in individuals at risk; serum phosphate monitoring is needed in those receiving more than one dose [27].

Various IV iron preparations and their typical adult dosages are detailed in **Table 1**.

3. Management of iron deficiency anemia in CKD

3.1 Principles of diagnosis in CKD stages 4 and 5

In patients with advanced CKD, iron deficiency is a frequent and complex contributor to anemia. The most important disruptor of iron availability in CKD is the hormone hepcidin. In CKD stages 4 and 5, systemic inflammation and reduced renal clearance lead to persistent elevation of hepcidin levels, which in turn suppresses iron mobilization and absorption. This hepcidin-driven block constitutes functional iron deficiency – a state where iron is present but unavailable for erythropoiesis (**Figure 1**). Simultaneously, absolute iron deficiency is also common, resulting from reduced dietary intake, uremia-related malabsorption, chronic gastrointestinal bleeding, or blood loss associated with dialysis. In patients receiving hemodialysis (HD), annual iron losses may reach up to 2–3 grams [29].

The diagnostic approach has relied on serum ferritin (reflecting iron stores) and transferrin saturation (TSAT, reflecting circulating iron availability), though both are imperfect as they are affected by inflammation. The Kidney Disease: Improving Global Outcomes (KDIGO) 2012 guidelines proposed diagnostic thresholds that remain in use (**Table 2**) [6]. Newer biomarkers, like reticulocyte hemoglobin

Iron IV	Elemental iron concentration	Dosage in adults	Skin test
Ferric carboxymaltose	50 mg/ml	≥50 kg: 1 or 2 doses of 750 mg, administered 7 or more days apart <50 kg: 1 or 2 doses of 15 mg/kg, administered 7 or more days apart	Not required
Ferric Isomaltoside	100 mg/ml	≥50 kg: Single dose of 1,000 mg or up to 3 doses of 500 mg given over 7 days <50 kg: Single dose of 20 mg/kg	Not required
Ferric gluconate	12.5 mg/ml	Multiple doses of 125–250 mg	Recommended if the patient has a history of multiple drug allergies
Ferumoxytol	30 mg/ml	Single dose of 1,020 mg or 2 doses of 510 mg, given 3 to 8 days apart	Not required
Iron dextran, low molecular weight (LMW ID)	mg/ml	Single dose of 1,000 mg (diluted in 250 mL of normal saline) given over 1 hour, or multiple doses of 100 mg	Yes, 25 mg (0.5 mL) prior to the first dose
Iron sucrose	20 mg/ml	Multiple doses of 100–300 mg	Recommended if the patient has a history of multiple drug allergies

Table 1.
Intravenous iron formulations used in adults. Adapted from Auerbach M. Treatment of iron deficiency and iron deficiency anemia in adults, 2025 [28].

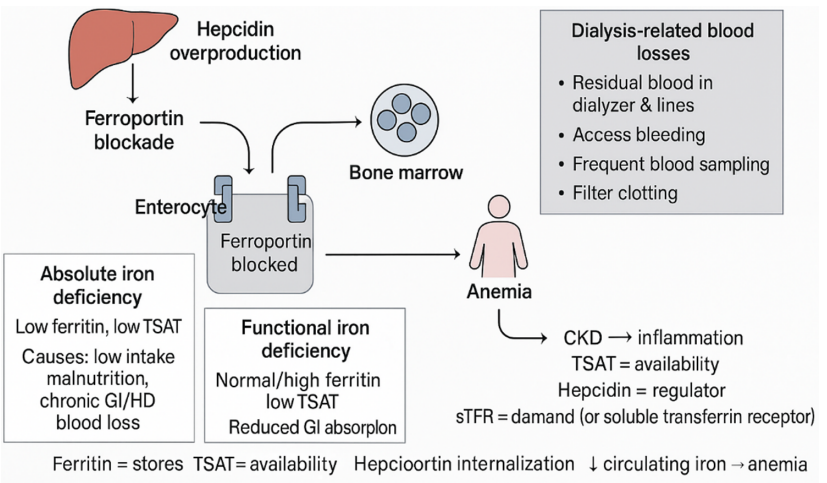


Figure 1.
Iron metabolism in advanced chronic kidney disease

content (CHr or Ret-He) and the percentage of hypochromic red cells (%HYPO), may offer better precision but are not yet widely adopted [13, 30].

Pathophysiology of absolute and functional iron deficiency in CKD is driven by hepcidin overproduction and dialysis-related blood losses, leading to anemia.

Patient GROUP	TSAT	Ferritin	Interpretation
CKD Stage 4 or 5 (nondialysis)	<20%	<100 ng/mL	Absolute iron deficiency
CKD Stage 4 or 5 (nondialysis)	<20%	≥100 ng/mL	Functional iron deficiency
CKD stage 5 on HD or PD	<20%	<200 ng/mL	Functional iron deficiency

Table 2.
Operational definitions of iron deficiency in CKD stages 4 and 5 (adapted from KDIGO, 2012).

3.2 Management in nondialysis patients (stage 4 and stage 5 CKD)

In patients not yet requiring dialysis, functional iron deficiency, driven by elevated hepcidin, is common. Oral iron has traditionally been the first-line therapy, but absorption is hindered in the uremic gut. When oral therapy fails, IV iron is the preferred option, as it is more effective at increasing hemoglobin levels and reducing ESA needs. KDIGO recommends initiating iron therapy if TSAT is <30% and ferritin is <500 ng/mL [31].

3.3 Management in HD patients: Lessons from the PIVOTAL trial

In HD patients, management is critical due to ongoing iatrogenic blood losses. The landmark PIVOTAL trial [7] compared a proactive, high-dose IV iron regimen (400 mg monthly unless ferritin >700 ng/mL or TSAT >40%) to a reactive, low-dose regimen. The proactive group had higher hemoglobin levels, lower ESA requirements, fewer transfusions, and reduced rates of hospitalization and cardiovascular events, with no increase in infections or serious adverse events (**Table 3**). These findings support a more liberal use of IV iron in HD patients, provided ferritin does not exceed ~700 ng/mL. While PIVOTAL established safety up to a ferritin of 700 ng/mL, observational data suggest ferritin values above 1,200 ng/mL may be associated with an increased mortality risk [11].

3.4 Management in peritoneal dialysis patients

In patients on peritoneal dialysis (PD), iron management is less studied. While these patients lose less blood than HD patients, inflammation and malnutrition can still lead to iron deficiency. Oral iron may be sufficient initially, but IV iron is often necessary in ESA-treated individuals. Protocols used in HD can be adapted to PD, with monthly monitoring of iron studies advisable in ESA users [32].

4. Management of iron in special situations

4.1 Iron in sepsis and active infections

4.1.1 Iron in severe sepsis or septic shock

For patients with severe sepsis or septic shock who have preexisting iron deficiency anemia, the consensus is to avoid IV iron during the acute phase of the infection. During sepsis, there is a rapid increase in hepcidin, leading to hypoferremia and iron

Outcome/endpoint	Proactive high-dose IV iron	Reactive low-dose IV iron	Effect estimate (95% CI)	P value	Direction
Monthly ESA dose (IU), median	29,757	38,805	Median difference −7,539 IU (approx.)	–	↓ ESA with proactive
Hemoglobin (Hb)	Faster rise to target 10–12 g/dL; maintained within target	Slower rise (needed more ESA)	Qualitative (per trial report)	–	–
Primary composite CV endpoint (time to first event: death, nonfatal MI, nonfatal stroke, or HF hospitalization)	320/1,093 (29.3%)	338/1,048 (32.3%)	**HR 0.85 (0.73–1.00)**	**0.04** (after NI met)	↓
Recurrent composite CV events (death + MI + stroke + HF hospitalization; total events)	429 events	507 events	Rate ratio 0.77 (0.66–0.92)	–	↓
All-cause death	246/1,093 (22.5%)	269/1,048 (25.7%)	HR 0.84 (0.71–1.00)	–	↓
Any hospitalization	–	–	HR 1.01 (0.90–1.12)	–	↔
Hospitalization for heart failure	–	–	**HR 0.66 (0.47–0.93)**	–	↓
Stroke	–	–	HR 0.79 (0.61–1.03)	–	↓ (ns)
Hospitalization for infection	–	–	HR 0.99 (0.82–1.16)	–	↔
All infection episodes (rate)	63.3 per 100 patient-years	69.4 per 100 patient-years	Rate ratio 0.91 (0.79–1.05)	–	↔
Any blood transfusion	–	–	**HR 0.79 (0.65–0.95)**	–	↓

Abbreviations: ESA, erythropoiesis-stimulating agent; CV, cardiovascular; HR, hazard ratio; MI, myocardial infarction; HF, heart failure.

Table 3.
PIVOTAL trial outcomes [7] (proactive high-dose IV iron vs. Reactive low-dose).

sequestration within cells. This is considered a host-protective response, as it limits iron availability for pathogens and reduces the risk of iron-catalyzed oxidative injury. Increased levels of nontransferrin-bound iron are associated with oxidative stress and worse outcomes in critically ill patients [33, 34]. Administering IV iron could theoretically "feed" the infection and worsen its course. Furthermore, free iron can catalyze the formation of free radicals, increasing oxidative stress and contributing to tissue damage. The recommendation is to stabilize the patient, treat the sepsis, and reevaluate the need for iron repletion once the acute infectious process has resolved [35, 36].

Iron preparation	Dose
Ferric carboxymaltose	750–1,000 mg observation for hypophosphatemia
Ferric derisomaltose	1,000 mg single dose
Ferumoxytol	510 mg twice or 1,020 mg once
Iron sucrose	200 mg 5 times or 300 mg 3 times weekly
Ferric gluconate |	125 mg 8 times, no closer than every 2 days
Erythropoiesis-stimulating agents (ESAs) with IV iron	Not routinely recommended in critically ill patients

Table 4.
Iron preparation and doses.

4.1.2 IV iron formulations for postsepsis use

For patients requiring iron supplementation after the resolution of septic shock, the IV route is preferred due to poor absorption of oral iron in the context of systemic inflammation [37]. The choice of IV formulation should be individualized. Alternatives to iron dextran include those listed in **Table 4**.

5. Conclusion

The management of iron deficiency anemia is a critical component of care for patients with advanced CKD, directly impacting morbidity, mortality, and quality of life. The therapeutic approach must be tailored, recognizing the limited efficacy of oral iron due to uremia-induced malabsorption and intolerance, which positions IV iron as the cornerstone of therapy, especially for the dialysis population. The findings from the PIVOTAL trial have established a new paradigm for HD patients, endorsing a proactive, high-dose IV iron strategy that improves cardiovascular outcomes and reduces ESA dependence, challenging previous conservative approaches. Nonetheless, clinicians must remain vigilant, individualizing treatment based on CKD stage and dialysis modality, while carefully navigating special situations like active infection, where delaying iron therapy is a key safety measure. Future efforts should focus on refining diagnostic tools beyond ferritin and TSAT and optimizing long-term iron supplementation strategies to ensure sustained benefits while minimizing potential risks.

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Chapter 7

Intrauterine Transfusion in the Treatment of Fetal Anemia

Berivoj Mišković and Ivana Omazić

Abstract

Intrauterine transfusion is a lifesaving procedure used to treat severe fetal anemia. This intervention is critical for preventing fetal damage and death, enabling pregnancies to continue until a viable gestational age. The most common cause of fetal anemia is the incompatibility between maternal and fetal erythrocyte antigens. Once the alloimmunization is determined and maternal titers have reached critical values, the severity of anemia is assessed using Doppler ultrasound measurements of peak systolic velocity in the middle cerebral artery. Values of 1.5 multiples of the median or higher are an indication for cordocentesis and potentially intrauterine transfusion. The most effective method of intrauterine transfusion, called intravascular transfusion, involves delivering donor blood directly into the umbilical vein between the 18th and 35th weeks of gestation. Donor blood must meet strict criteria, including being type "O," Rh-D negative, and gamma-irradiated to prevent infections and immune reactions. It must be cytomegalovirus antibody-negative, with leucodepletion performed to further reduce the risk of transmission. Freshly prepared, high-hematocrit blood ensures optimal oxygen delivery in the smallest possible volume. The required volume of donor blood is calculated based on the initial fetal hematocrit, fetal weight, donor blood hematocrit, and the target fetal hematocrit. Advancements in the prevention and diagnosis of Rh-D alloimmunization, as well as the management and treatment of severe fetal anemia, are the result of a close collaboration between fetal medicine and transfusion medicine specialists. The multidisciplinary approach has led to a significant improvement in prenatal care.

Keywords: Rh-alloimmunization, fetal anemia, donor blood preparation, intrauterine transfusion, intravascular transfusion

1. Introduction

According to etiopathogenesis, fetal anemias (FA) are classified as either immunological or non-immunological.

Immunological FA is a result of incompatibility between maternal and fetal erythrocyte antigens, with the Rh-D antigen incompatibility being the most common one.

Non-immunological FA is caused by Parvovirus B-19 infection, α-thalassemia, fetomaternal hemorrhage, twin-to-twin transfusion syndrome, vascular tumors, and arteriovenous malformations.

Prophylactic use of Rh immune globulin (RhIG) reduced the number of fetal anemias caused by Rh-D antigens substantially. However, effective methods for the prevention of fetal anemia caused by non-D antigens, such as Kell, as well as those of non-immune etiology, for example, anemia resulting from Parvovirus B-19 infection, are yet to be established.

The introduction of non-invasive prenatal diagnosis of fetal Rh-D status using cell-free fetal DNA analysis, as well as Doppler ultrasound assessment of the severity of fetal anemia, has enabled the avoidance of invasive procedures in the diagnosis of alloimmunization and severe fetal anemia. These advancements have significantly improved the safety of prenatal management by reducing the risks associated with invasive techniques such as amniocentesis and cordocentesis.

At this moment, intrauterine transfusion (IUT) is the only effective treatment for severe fetal anemia, preventing fetal damage and intrauterine demise while allowing pregnancy to be prolonged until viable gestational age.

2. Fetal circulation and red blood cells

Fetus receives all the necessary oxygen and nutrients through the umbilical cord. Umbilical cord is comprised of two umbilical arteries and one umbilical vein. Umbilical vein delivers oxygenated blood from the placenta to the fetus, while umbilical arteries return deoxygenated blood back to the placenta.

Optimal oxygen supply to the fetal tissues is obtained via increased fetal heart rate, increased number of erythrocytes per unit, greater oxygen-binding affinity of erythrocyte due to fetal hemoglobin F, and a larger erythrocyte volume.

Hemoglobin levels increase throughout pregnancy, from 100 to 110 g/L in the second trimester to 140–150 g/L at term, with a standard deviation of 12 g/L.

Moderate fetal anemia is present when fetal hemoglobin levels fall between 2 and 4 standard deviations below the median for a given gestational age. Levels that are 4 standard deviations below the median for a given gestational age indicate severe fetal anemia [1].

3. Causes of fetal anemia

As previously mentioned, fetal anemias are classified as either immunological or non-immunological.

Parvovirus B-19 infection is the most common cause of non-immune fetal anemia, which occurs due to viral erythropoiesis suppression. Many other pathological conditions, including some fetal aneuploidies, inherited diseases such as α-thalassemia, fetomaternal hemorrhage, vascular tumors, and arteriovenous malformations, are described as a cause of fetal anemia but are far less represented [2].

Maternal alloimmunization to fetal erythrocyte antigens is the most common cause of immune fetal anemia. In most cases, sensitization occurs before pregnancy, affected by fetal anemia, either by transfusion of blood products containing mismatched red blood cell antigens or during the course of a previous pregnancy.

Incompatibility between fetal and maternal erythrocyte antigens activates the maternal immune system and triggers production of IgM and, later, IgG antibodies. Neonatal Fc receptor in the placenta enables IgG antibodies to pass the placental barrier and bind to fetal erythrocytes, leading to extravascular hemolysis in the fetal

spleen. As a result, some fetuses will develop severe fetal anemia, eventually leading to fetal hydrops.

Alloimmunization occurs mostly due to RhD incompatibility, but other antigens such as Kell and Rhc can also cause severe fetal anemia. Kell antibodies have the ability to suppress erythrocyte precursors and therefore cause severe fetal anemia without features associated with hemolysis of mature erythrocytes.

Systematic antenatal and postpartum administration of Rh immune globulin has led to a significant decrease in the number of cases of Rh-alloimmunization in pregnancy and hemolytic disease of the fetus and newborn (HDFN). However, a substantial number of cases are still reported.

4. Prevention of Rh-alloimmunization

American College of Obstetricians and Gynecologists recommends prophylactic use of anti-D immune globulin in all unsensitized Rh D-negative women at 28 weeks of gestation, regardless of the presence of potentially sensitizing events.

Postnatally, if the umbilical cord blood sampling confirms Rh D positive status of the fetus, all Rh D-negative unsensitized women should receive anti-D immune globulin within 72 hours.

Events such as miscarriage, ectopic pregnancy, molar pregnancy, antenatal hemorrhage, abdominal trauma, second and third trimester fetal demise, as well as procedures such as chorionic villus sampling, amniocentesis, external cephalic version, and pregnancy termination are potentially sensitizing.

Although the risk of alloimmunization is low, the consequences can be significant. Therefore, the administration of Rh D immune globulin is recommended in unsensitized Rh D-negative women when the fetuses could be Rh D positive [3].

5. Diagnosis of Rh-isoimmunization

Indirect Coombs test (ICT) is an antibody screen test obtained at the first prenatal visit. If the test returns positive for an antibody associated with severe fetal anemia, and paternity is certain, paternal zygosity is determined. In case of a heterozygous genotype or uncertain paternity, cell-free fetal DNA testing for determining RhD status of the fetus is now available as early as 10 weeks of gestation. Since the introduction of cell-free fetal DNA testing, invasive methods have been abandoned in favor of non-invasive techniques.

Following positive ICT for an antibody associated with FA and HDFN, the maternal serum titer should be determined. Maternal titer values are reported as the final serum dilution of the indirect Coombs test [4].

A critical titer is the maternal antibody titer level at which there is a significant risk for severe fetal anemia and is considered to be 1:16 for anti-D and 1:4 for anti-Kell and anti-c [4, 5].

Anti-D titers can predict the severity of fetal and neonatal hemolytic anemia, especially in primo immunization cases, whereas titer values in anti-Kell and anti-c immunization do not necessarily correlate with disease severity.

Maternal titer values are determined monthly until 24 weeks of gestation and every 2 weeks after. Once the critical titer is reached, serial Doppler ultrasound measurements of middle cerebral artery peak systolic velocity (MCA-PSV) is a method of further fetal surveillance [4].

6. Assessment of severity of fetal anemia

The technique of Doppler ultrasound measurement of middle cerebral artery peak systolic velocity for the prediction of the severity of fetal anemia was first introduced by Mari et al. Authors have found that the hemoglobin levels directly correlate with the MCA-PSV and have suggested that the values of 1.5 multiples of median or more for a given gestational age should be used as a threshold for severe fetal anemia [6].

Increased peak systolic velocities are a result of hemodynamic changes resulting from severe FA, such as centralization of circulation and dilatation of cerebral blood vessels.

Measurements of MCA-PSV are initiated once the critical titer is reached, or as early as 15 weeks of gestation in case of an alloimmunization in a previous pregnancy. Measurements are repeated weekly. A peak systolic velocity in the middle cerebral artery exceeding 1.5 multiples of the median is an indication for cordocentesis to determine fetal hematocrit levels. Based on the obtained hematocrit value, an intrauterine transfusion may be administered.

A good understanding of measurement techniques is of critical importance since it can significantly affect the obtained values. Therefore, measurements should be performed by a trained specialist to avoid under- or overestimation of the degree of fetal anemia [7].

7. Intrauterine transfusion

Intrauterine transfusion is currently the only effective treatment for severe fetal anemia. IUT is performed if the cordocentesis reveals fetal hemoglobin levels 2 standard deviations below the mean for gestational age or if fetal hematocrit is below 30 percent [1, 8]. There are two types of intrauterine transfusion, intraperitoneal transfusion and intravascular transfusion.

7.1 Intraperitoneal transfusion

Intraperitoneal intrauterine transfusion is a procedure in which red blood cells are transfused directly into the fetal peritoneal cavity. From there, the fetal lymphatic system gradually absorbs the transfused blood into the circulation.

This method is rarely used due to poorer results compared to intravascular intrauterine transfusion. However, in cases where the puncture of the umbilical cord is impossible, especially in the earlier gestational ages, intraperitoneal transfusion may still be performed until conditions for intravascular transfusion have been met [9, 10].

7.2 Intravascular transfusion

Intravascular intrauterine transfusion is an effective method for delivering donor blood directly into the fetal circulation. The optimal puncture site is at the placental insertion of the umbilical cord, with the primary goal being to access the umbilical vein. If direct access to the placental insertion is not feasible, alternative sites include the free-floating portion of the umbilical cord or the intra-abdominal segment of the umbilical vein.

IUT is typically performed between the 18th and 35th week of gestation. Before 20 weeks, the procedure is technically highly challenging due to the small size of fetal

blood vessels [11]. After 35 weeks of gestation, delivery followed by postnatal transfusion therapy is more appropriate than attempting further intrauterine transfusions.

7.3 Preparation of donor blood

Besides the usual mandatory test required for blood donation, donor blood must meet additional strict criteria.

Blood must be type "O," RhD-negative, and free from any clinically significant irregular blood group antibodies and high-titer anti-A and/or anti-B. The sample is crossmatched to the maternal sample to prevent the formation of maternal antibodies from the IUT.

It must be negative to cytomegalovirus antibody, as well as leucocyte-depleted to further reduce the risk of cytomegalovirus transmission.

It is recommended for blood to be stored no longer than 5 days from the donation to keep the levels of 2, 3-diphosphoglycerate high, which secures high oxygen-binding capacity.

The sample is irradiated with 25 Gy of gamma irradiation to prevent infections and immune reactions such as graft-versus-host reaction, and should be used within 24 hours from the irradiation.

Blood is washed and concentrated to a hematocrit of 75–80%. Freshly prepared, high-hematocrit sample ensures optimal oxygen delivery in the smallest possible volume. This minimizes the risk of volume overload of the fetus.

The required volume of donor blood is calculated based on the initial fetal hematocrit, fetal weight, donor blood hematocrit, and the target fetal hematocrit [12, 13].

7.4 Technique of intrauterine transfusion

Described technique is the one used in the Clinical hospital "Sveti Duh," Zagreb, Croatia, and is in accordance with relevant guidelines.

Ultrasound-guided technique is used to target the umbilical vein, which has a wider lumen and lacks a muscle layer. The use of the umbilical artery carries a higher risk of spasm and subsequent complications.

Puncture site is at the placental insertion of the umbilical cord. If access to the placental insertion is not available, a free-floating portion of the umbilical cord is used. Free loop puncture hold a higher risk of needle luxation. Puncture at the intra-abdominal segment of the umbilical vein is possible but rarely used due to the higher risk of complications. The intravascular position of the needle is confirmed by blood aspiration.

We use a freehand technique without needle-guiding devices or assistants.

After the cordocentesis, we take the initial sample of fetal blood, which is then processed in the automated hemocytometer, and levels of hemoglobin and hematocrit are determined. Mean corpuscular volume (MCV) of red blood cells higher than 100 ml suggests that fetal, as opposed to maternal, erythorcytes have been obtained. Based on the obtained values of fetal hematocrit, fetal weight, donor blood hematocrit, and target fetal hematocrit, the required volume of donor blood is calculated.

After taking the initial sample of fetal blood, if necessary, we administer a short-acting paralytic agent, usually vecuronium in the dose adjusted by fetal weight (0.1 mg per hg of estimated fetal weight), to minimize fetal movements and facilitate successful completion of the transfusion [14].

During the transfusion, we usually take three samples of fetal blood, the previously mentioned initial one to determine fetal hematocrit, one during the procedure, and one at the end of the procedure to assess the effect of transfusion and to plan further IUT. Fetal heart rate is periodically monitored.

7.5 Timing of subsequent transfusions

There is no consensus on the optimal timing of repeated transfusions. Some authors empirically schedule repeated transfusions at intervals ranging from 10 days to 4 weeks, depending on the number of previous transfusions. Another approach is to time repeated transfusions based on the expected decline in fetal hemoglobin levels. It is estimated that hemoglobin decreases by 0.4 g/dL/day after the first transfusion, 0.3 g/dL/day after the second, and 0.2 g/dL/day after the third. Following multiple IUTs, a larger proportion of fetal blood is composed of donor erythrocytes, which are not at risk of hemolysis. Due to altered blood flow dynamics in the middle cerebral artery in the presence of donor erythrocytes, the use of MCA-PSV for the timing of repeated IUTs is unreliable [15–17].

7.6 Complications and outcomes of intrauterine transfusion

Although intrauterine transfusion is considered relatively safe and is among the safest fetal treatment procedures, complications continue to occur. Most frequent complications are transient fetal bradycardia and bleeding from the puncture site.

In the large retrospective study from the Netherlands, in a total of 1678 IUTs, 69 complications were reported, from which 44 were considered to be directly procedure related (PR). Overall complication rate was 1.2 percent.

The incidence of PR complications significantly declined over time, presumably due to increased experience. The risk of PR perinatal loss also decreased over time, from 1.6% to 0.6% per procedure. Overall perinatal survival reported in this study was 97.0% from 2001 onwards.

Reported complications were preterm prelabor rupture of membranes (0.1 percent) and preterm delivery, infection (0.1 percent), emergency Cesarean section (0.4 percent) due to fetal distress, and fetal or neonatal death [18].

The largest long-term follow-up study to evaluate the incidence and risk factors for neurodevelopmental impairments in children treated with IUT is the LOTUS study (long-term follow-up after intrauterine transfusions). The aim of the study is to determine the incidence of long-term neurodevelopmental impairment and associated perinatal risk factors in children treated with IUTs prenatally. So far, this study has found that hydrops was the most important risk factor for neurodevelopmental impairment [19].

This study, as well as other currently available studies, confirms that the benefit of intrauterine transfusion outweighs the potential risks.

8. Conclusion

Advancements in the treatment of fetuses with severe anemia are the result of progress at multiple levels, including prevention, diagnosis, and therapy.

The introduction of RhD immunoprophylaxis has significantly reduced the number of reported cases of RhD alloimmunization, while the development of

non-invasive fetal diagnostic techniques has facilitated the determination of fetal genotype. Additionally, the implementation of ultrasound-based assessment of fetal anemia severity has decreased the need for invasive diagnostic procedures and subsequent complications.

Improvements in intrauterine transfusion techniques have led to a reduction in complications and an increase in fetal survival rates.

All these advancements are the result of a multidisciplinary approach, primarily involving specialists in fetal medicine, transfusion medicine, and genetics.

Conflict of interest

The authors declare no conflict of interest.

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