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## REVIEW

# Iron in blood cells: Function, relation to disease, and potential for magnetic separation

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## Abstract

Iron in blood cells has several physiological functions like transporting oxygen to cells and maintaining iron homeostasis. Iron is primarily contained in red blood cells (RBCs), but monocytes also store iron as these cells are responsible for the recycling of senescent RBCs. Iron also serves an important role related to the function of different leukocytes. In inflammation, iron homeostasis is dependent on cytokines derived from T cells and macrophages. Fluctuations of iron content in the body lead to different diseases. Iron deficiency, which is also known as anemia, hampers different physiological processes in the human body. On the other hand, genetic or acquired hemochromatosis ultimately results in iron overload and leads to the failure of different vital organs. Different diagnoses and treatments are developed for these kinds of disorders, but the majority are costly and suffer from side effects. To address this issue, magnetophoresis could be an attractive technology for the diagnosis (and in some cases treatment) of these pathologies due to the paramagnetic character of the cells containing iron. In this review, we discuss the main functions of iron in blood cells and iron-related diseases in humans and highlight the potential of magnetophoresis for diagnosing and treating some of these disorders.

## KEYWORDS

anemia, iron, magnetophoresis, red blood cells (RBC)

## 1 | INTRODUCTION

Iron is one of the most vital micronutrients in human organisms as it plays an important role in oxygen transport, nucleic acid replication and repair, host defense, cellular proliferation, oxidative metabolism, and many catalytic reactions (Camaschella, Nai, et al., 2020; Yiannikourides & Latunde-Dada, 2019). Approximately 75% of the iron in the human body is associated with erythrocytes as hemoglobin (Hb), a molecule composed of four units, each containing one heme group and one protein chain, which transports oxygen (Gupta, 2014; Nagababu et al., 2008). Part of the remaining iron in the human body is present as free iron, which has the potential to become cytotoxic and acts as a catalyst in the production of reactive oxygen species (ROS), such as superoxide ( $O_2^{\bullet-}$ ), hydroxyl ( $OH^{\bullet}$ ),

hydrogen peroxide ( $H_2O_2$ ), and hypochlorous acid (HOCl); these are linked to various kind of diseases like cancer, insulin resistance, diabetes mellitus, and cardiovascular diseases (Alfadda & Sallam, 2012; Bayr, 2005; Soares & Hamza, 2016). The mechanism of iron homeostasis maintains an adequate iron level in the body, avoiding accumulating iron in excess and limiting its uptake from the environment (Camaschella, 2019). Leukocytes have different roles in iron homeostasis. For example, monocytes can uptake iron through different pathways and control its export (Sukhbaatar & Weichhart, 2018; Weiss, 2002). On the other hand, iron is needed for the correct function of leukocytes like T cells, B cells, natural killer (NK) cells, and neutrophils (Cronin et al., 2019). During an infection, the functions of iron uptake, storage, and removal in cells and tissues are controlled by the secretion of cytokines (Ganz & Nemeth, 2015).

Cytokines derived from T cells and monocytes regulate cellular iron homeostasis by affecting the expression of proteins involved in the uptake and storage of iron molecules (Ludwiczek et al., 2003).

Both iron deficiency and excess iron are harmful to the human body and may lead to different disorders, like anemia and hemochromatosis, respectively. In anemia, the concentration of Hb is insufficient to meet the human's physiological needs and affects roughly one-third of the world's population (Chaparro & Suchdev, 2019). The most common cause of anemia is iron deficiency, which affects mainly growing children, and premenopausal and pregnant women (Cappellini et al., 2020). Disturbance of iron homeostasis can also cause anemia of chronic disease (ACD), in which uptake and retention of iron increase in the cells of the reticulo-endothelial system and thus, limit the availability of iron for erythropoiesis (Weiss & Goodnough, 2005). Autosomal recessive disorders like thalassemia and sickle cell disease can also lead to anemia (Huang et al., 2020; Muncie HL & Campbell, 2009; Xu & Thein, 2019). Deficiency of vitamins like vitamins A, B12, C, E, riboflavin, and folic acid is also linked to anemia (Fishman et al., 2000). Differently, hemochromatosis is a group of disorders in which intestinal iron absorption becomes uncontrolled and leads to iron overload, which can cause arthritis, diabetes, heart failure, hepatic cirrhosis, and hepatocellular carcinoma (Girelli et al., 2022). Causes of hemochromatosis may be genetic or acquired, like frequent blood transfusions or excess dietary intake of iron (Chacon et al., 2013). Considering the importance of iron in several different physiological processes in the body, research on iron functions and iron-related diseases in the human body is constantly needed.

Because of the magnetic character of iron, some blood cells (e.g., deoxyhemoglobin red blood cells (RBCs) and some monocytes) show paramagnetism instead of diamagnetism (Furlani, 2007; Gómez-Pastora et al., 2021). This means that these cells are susceptible to manipulation using magnetic fields. As such, magnetophoresis can serve as an attractive technique for the diagnosis and treatment of iron-related diseases. Magnetophoresis, a term used as an analogy to electrophoresis, describes particle and/or cell motion in a viscous medium under the influence of magnetic fields (Gómez-Pastora, Moore et al., 2022; Zborowski et al., 2002). Magnetophoretic-based techniques are presented as simple, low-cost, and portable technologies that can be used to manipulate biological material, in comparison to other methods for blood cell manipulation such as electrophoresis, thermophoresis, dielectrophoresis, optical trapping, and acoustophoresis (Karampelas & Gómez-Pastora, 2022; Zhu & Trung Nguyen, 2010). Even at a low volume fraction, the paramagnetic constituents of a cell (in this case, iron) control the cell's volume magnetic susceptibility, as they have a high magnetic susceptibility relative to the diamagnetic constituents of the cell (Xue et al., 2019). Therefore, magnetophoresis can be a promising tool to diagnose and treat different blood diseases (Bessis & Weed, 1973; D'alessandro et al., 2017). Indeed, recent studies have shown how magnetophoresis can be applied not only for blood cell separation but also for diagnosing and treating different blood disorders. For example, Chalmers et al. and Kim et al. presented a

novel method in which the RBC magnetic properties (i.e., magnetic susceptibility) can be used to detect iron deficiency anemia (IDA) via magnetophoresis (Chalmers et al., 1999; Kim, Gómez-Pastora, Gilbert, et al., 2019). The studies of McCloskey et al. and Munaz et al. evaluated the possibility of using magnetic fields for the separation of healthy and unhealthy blood cells (McCloskey et al., 2003; Munaz et al., 2018). Moreover, other works have also presented how permanent magnet-based devices could be used for the diagnosis of other anemias like sickle cell disease (Ge & Whitesides, 2018; Kumar, Patton, et al., 2014; M. Weigand, Pastora, Palmer, et al., 2022; M. Weigand, Gomez-Pastora, Strayer, et al., 2022).

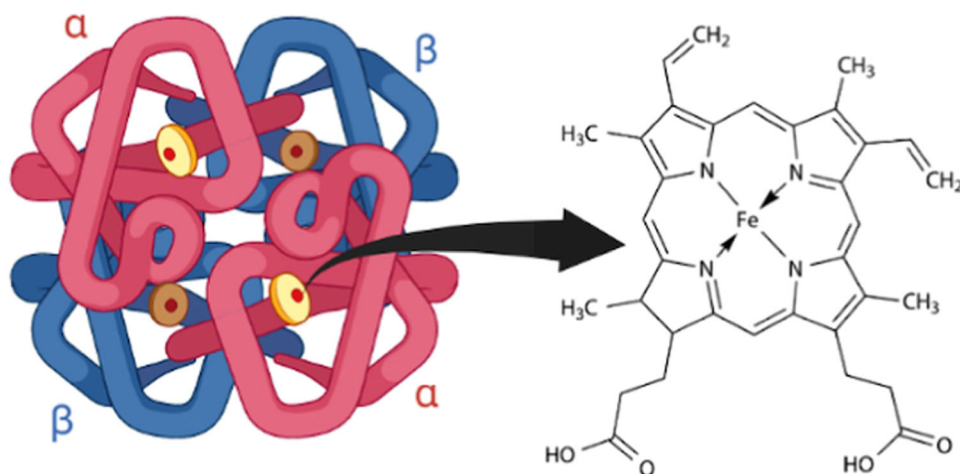
This review is structured as follows. After this introduction, Section 2 describes the main functions of iron in RBCs and white blood cells (WBCs) through a more detailed discussion of the previous paragraphs. In Section 3, we present a comprehensive characterization of the various types of diseases linked to unbalanced levels of iron in blood cells. Next, Section 4 brings the fundamentals of magnetophoresis and its potential application to the diagnosis and treatment of blood-related medical conditions. This review finishes with the main conclusions obtained from gathering the current knowledge of this promising field of study.

## 2 | FUNCTION OF IRON IN BLOOD CELLS

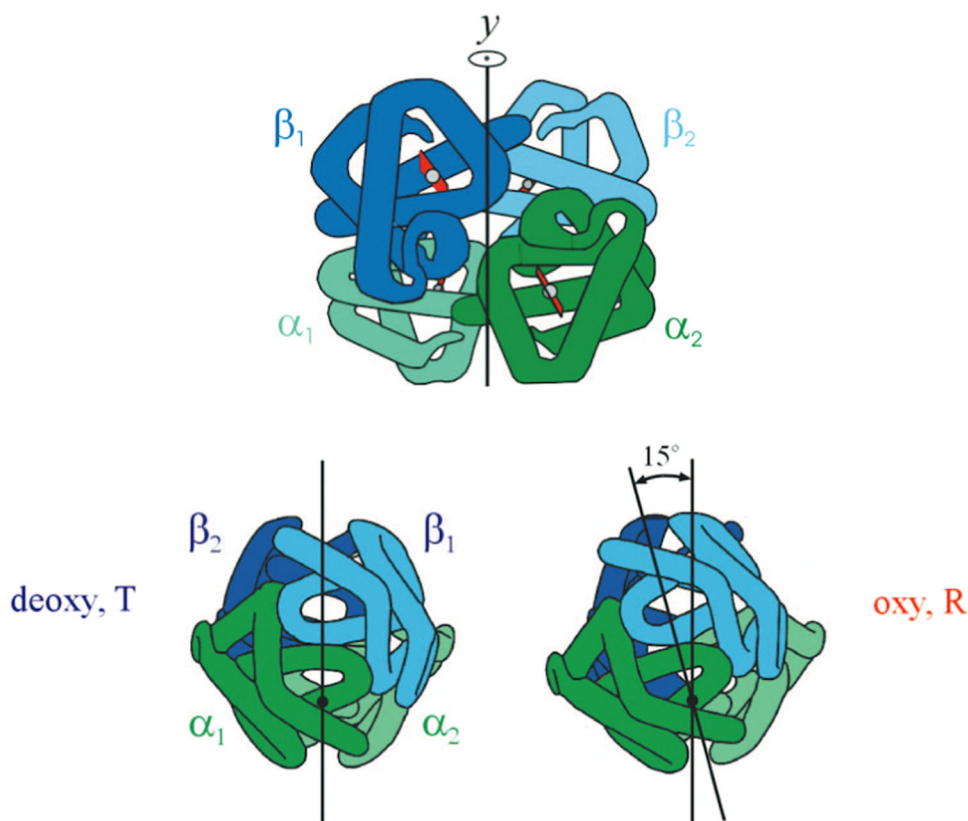
### 2.1 | Iron in RBCs

RBCs, also known as erythrocytes, play the main role of transporting  $O_2$  from the lungs to other cells and tissues and  $CO_2$  back from cells/tissues to the lungs. Human RBCs have a biconcave disc shape and red color because of the large amount of Hb in their cytoplasm (Bessis & Weed, 1973). Hb is the protein responsible for the transport of  $O_2$  and  $CO_2$ . Every RBC contains around 270 million Hb molecules (D'alessandro et al., 2017). Hb consists of four polypeptide globin chains and every chain contains one non-protein or prosthetic heme group that binds reversibly to  $O_2$  (Perutz, 1979; Thomas & Lumb, 2012). Each Hb molecule is structured by two  $\alpha$  and two  $\beta$  polypeptide chains as seen in Figure 1 (Jensen et al., 1998). The heme group is composed of a ringlike porphyrin to which an iron atom is attached (Perutz, 1979). The iron atom's coordination number is 6, which means that it can receive 6 pairs of electrons (Ha & Bhagavan, 2015). Iron receives five electron pairs from the four nitrogen atoms of the porphyrin and another pair from the nitrogen in the histidine (Jensen et al., 1998). The remaining is used for transporting  $O_2$  or  $CO_2$  (Ha & Bhagavan, 2015).

Hb is very efficient in the transport of  $O_2$  and  $CO_2$ . It can transport up to 50 times the  $O_2$  amount that can be delivered to adults artificially (Klinken, 2002). The valence electron of iron in the heme group remains unchanged during oxygenation and deoxygenation of Hb (Gordon-Smith, 2013; Jensen et al., 1998; Perutz, 1979). The deoxygenated heme group is referred to as the tense state (T-state) and the oxygenated heme group is referred to as the relaxed state (R-state) (Ha & Bhagavan, 2015; Jensen et al., 1998;



**FIGURE 1** Simplified hemoglobin with heme groups and its molecular structure.



**FIGURE 2** T and R states of hemoglobin. Adapted from Eaton et al. (2007).

Perutz, 1979). The allosteric model describes the transition between these states of the heme group (Gordon-Smith, 2013; Monod et al., 1965). When the first oxygen molecule binds to the T-state heme group, its structure dramatically changes, and the  $\alpha$  and  $\beta$  chains rotate at about  $15^\circ$  angle relative to each other (Gordon-Smith, 2013; Jensen et al., 1998; Perutz, 1970), as presented in Figure 2. This rotation loosens the salt bridge between them and thus, it becomes R-state (Perutz, 1970). This state helps to bind the four oxygen molecules to each Hb unit (Ha & Bhagavan, 2015).

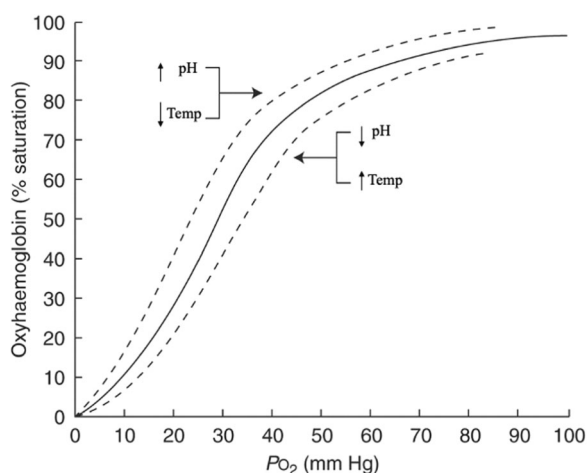
The transport of  $O_2$  is also governed by the partial pressure of oxygen ( $pO_2$ ) in the solution and the pH (Gordon-Smith, 2013). The collection of  $O_2$  molecules by Hb in the lungs is favored by a high partial pressure ( $pO_2$ ) and a high pH (Ha & Bhagavan, 2015). For this reason, it can bind to  $O_2$  easily and the Hb- $O_2$  dissociation curve, which is a curve that relates oxygen saturation and  $pO_2$  in blood (Ha & Bhagavan, 2015; Scrima et al., 2019), shifts left (Mairbäurl, 2013), as in Figure 3. When it reaches the cell/tissue where  $O_2$  is delivered,  $O_2$  is released because of the low  $pO_2$  and low pH; the disassociation



curve of oxygen shifts right (Mairbäurl, 2013). This is called the Bohr effect (Ha & Bhagavan, 2015).

## 2.2 | Iron in white blood cells

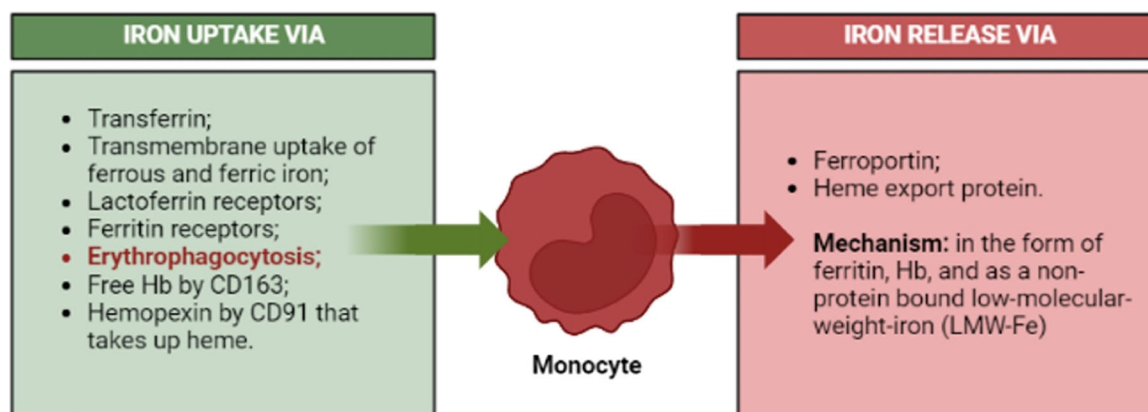
WBCs, also known as leukocytes, are produced in the bone marrow and found in the blood and lymph tissue (Institute N.C., 2022). The normal concentration of WBCs is 4000–10,000 cells per  $\mu\text{L}$  of blood (Blumenreich, 1990). Their main goal is to help the body fight against infections and other diseases through phagocytosis. For this reason, they are an integral part of the immune system (Institute N.C., 2022). Based on the presence of granules in their cytoplasm, WBCs can be classified into two types: granulocytes and agranulocytes. Granulocytes include basophils, eosinophils, and neutrophils, while agranulocytes include lymphocytes (T cells, B cells, and NK cells) and monocytes (LaRosa & Orange, 2008; Sahlol et al., 2020), the latter being an integral part of the recycling of iron in the blood.



**FIGURE 3** Hb- $\text{O}_2$  dissociation curve. Adapted from Walsh et al. (2006).

Every day, approximately 200 billion Hb molecules are produced in the human body. To produce this amount, the human body requires 20 mg of iron, but only 1–2 mg of iron is absorbed daily in the guts (Camaschella & Girelli, 2020; Hentze et al., 2004). Monocytes (and macrophages) play a major role in this regard by phagocytosing senescent RBCs and supplying iron to the bone marrow, thus being critical in iron recycling and iron homeostasis (Moura et al., 1998). They are the largest WBCs and approximately, 2%–8% of leukocytes are monocytes. Their kidney-shaped nucleus distinguishes them from other WBCs (Ashton, 2007). Monocytes are derived from the bone marrow and spend approximately 24 h in the circulation. After that, they enter the tissue and differentiate to become macrophages (Kim, Gómez-Pastora, Weigand, et al., 2019). Human monocytes are classified based on the expression of CD14 and CD16 receptors: classical ( $\text{CD14}^+$  and  $\text{CD16}^-$ ), intermediate ( $\text{CD14}^+$  and  $\text{CD16}^+$ ), and nonclassical ( $\text{CD14}^-$  and  $\text{CD16}^+$ ) monocytes (Ziegler-Heitbrock et al., 2010). Monocytes use different mechanisms to acquire iron: transferrin-mediated iron uptake, transmembrane uptake of iron ions, iron uptake by lactoferrin receptors, ferritin receptors, or via erythrophagocytosis (Weiss, 2002). Different pathways for iron uptake and iron release are represented by a schematic diagram in Figure 4. In some pathological conditions, nontransferrin-bound iron (NTBI) and free heme accumulate in the serum, which can lead to organ and cell damage and can be easily utilized by microbes (Haschka et al., 2019). In this regard, classical and intermediate monocytes can uptake NTBI and damaged erythrocytes by the CD163 receptor (Haschka et al., 2019; Rubio-Navarro et al., 2015). Monocytes also express CD91 (Hudig et al., 2014), which can capture heme by the iron-binding protein hemopexin (Hvidberg et al., 2005). Besides, ferroportin, which is the only iron exporter known to date (Hentze et al., 2004), is also expressed in macrophages/monocytes (Kim, Gómez-Pastora, Weigand, et al., 2019). That indicates that monocytes can also release iron, making it available for erythropoiesis (Kim, Gómez-Pastora, Weigand, et al., 2019).

Furthermore, hepcidin, which is produced in the liver in response to iron overload and inflammation (Ganz & Nemeth, 2006), controls iron export in monocytes (Weiss, 2009). Hepcidin binds to ferroportin



**FIGURE 4** Mechanisms of iron uptake and release by monocytes.

and degrades it (Nemeth et al., 2004), and thus, decreases iron export from the cell (De Domenico et al., 2007). On the other hand, if plasma iron decreases, it leads to a reduction in hepcidin synthesis, increasing iron delivery to plasma (De Domenico et al., 2007). Iron uptake and release in monocytes are controlled by different steps which can be thwarted by different diseases like hemochromatosis (Kim, Gómez-Pastora, Weigand, et al., 2019). Moreover, different receptors are expressed on monocytes by which they can sense any infection or tissue inflammation (Yang et al., 2014). If monocytes sense any damage or infection in tissue cells, they get rapidly recruited to the damaged or infected tissue and there they become macrophages (Yang et al., 2014). Macrophages can phagocytize three times more erythrocytes than monocytes (Weiss, 2009).

Also, iron is essential for producing ROS. ROS are byproducts of cellular metabolism that have different physiological functions in the body (Bardaweel et al., 2018) and are produced in neutrophils using iron (Barbusiński, 2009; Bystrom et al., 2014; Renassia et al., 2020). Neutrophils can also secrete lipocalin-2 and lactoferrin, which chelate iron in the case of iron increase in the body (Flo et al., 2004; Nakashige et al., 2015). Additionally, lymphocytes (B, T, and NK cells) need iron to perform their functions. NK cells' activation and activity against viruses are solely dependent on sufficient iron absorption (Littwitz-Salomon et al., 2021). B cells, which produce antibodies against bacteria (Althuwaiqeb & Bordoni, 2021), need iron for maturation (Jiang et al., 2019). Finally, the function and differentiation of T cells are solely dependent on iron (Bonaccorsi-Riani et al., 2015). Activation of T cells depends on the acquisition of iron through the transferrin receptor 2 (TfR-2) via the interleukin-2 (IL-2) pathway (Macedo et al., 2004; S. H. Ross & Cantrell, 2018). There are two main subsets of T cells: CD4+ T helper cells (Th cells), and CD8+ cytotoxic T cells (Sauls et al., 2018). Five major CD4+ Th cells have been identified to

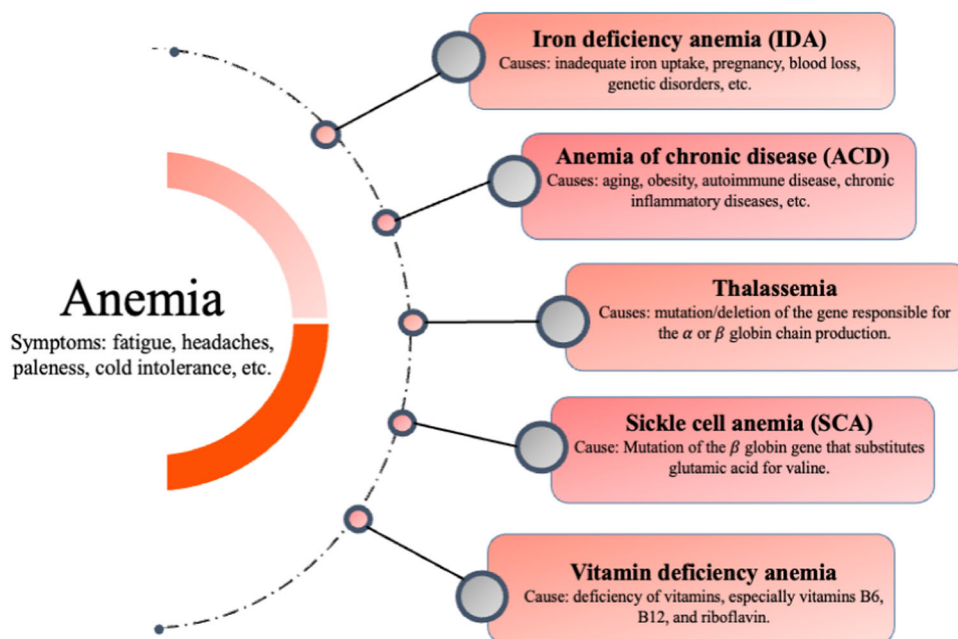
date (Th1, Th2, Th17, T<sub>reg</sub> (T regulatory), and Tfh (follicular T helper)), whereas CD8+ T cells differentiate into stem cell memory cells (T<sub>SCM</sub>), T central memory cells (T<sub>CM</sub>), T effector memory cells (T<sub>EM</sub>) and T effector cells, (T<sub>EFF</sub>) (Golubovskaya & Wu, 2016). Even though iron inhibits differentiation, iron oxide adjuvant increases the activity of Th1 cells (Knobloch et al., 2020; Neto et al., 2018). A similar effect of iron has been found for Th2 cells (Cronin et al., 2019). On the other hand, there is a disagreement about iron's effect on Th17 cells (Ni et al., 2022). Upregulation of transferrin receptor 1 (TfR-1) increases intracellular iron transport and leads to T<sub>reg</sub> cells to death (Feng et al., 2021). Moreover, iron promotes the differentiation of CD8+ cytotoxic T cells (Wang et al., 2021).

As presented above, iron is an important player in immunosurveillance because it has a pivotal role in differentiating immune cells and can interfere with the cytokines secreted by these cells (Hassan et al., 2016). Also, some leukocytes like monocytes are crucial for maintaining correct iron levels in the body. These cells uptake most of the iron from RBCs and are responsible for releasing it for future erythropoiesis. Therefore, even though iron is mostly associated with RBCs, the presence of the metal in WBCs and the effect that it poses on the immune system cannot be neglected.

### 3 | DISORDERS CHARACTERIZED BY ABNORMAL IRON LEVELS IN BLOOD CELLS

#### 3.1 | Anemia

The most prevalent condition characterized by low iron levels in the blood is anemia. There are different types of anemia, as schematized in Figure 5. In this subsection, the main types will be briefly explained



**FIGURE 5** Different types of anemia and respective symptoms and causes.

and the relationship between the iron levels in the cells and the development of the disease will be introduced.

### 3.1.1 | IDA

IDA is the most common anemia and most prevalent nutritional deficiency in the world (Camaschella, 2015; Lopez et al., 2016; Miller, 2013). This disease arises when the iron stores are not enough to support the normal production of erythrocytes (Miller, 2013). Individuals with low Hb levels in the blood (<13 g/dL for males and <12 g/dL for females), low transferrin saturation (<20%), and low ferritin concentrations (<30 ng/mL) should be evaluated for IDA (Cook, 2005; Johnson-Wimbley & Graham, 2011). Globally, there were 1.2 billion cases of IDA diagnosed in 2016 (Pasricha et al., 2021). According to the World Health Organization (WHO), the prevalence of anemia in the developing world is 39% for adults, 48% for children (ages from 5 to 14), and 52% for pregnant women, with IDA accounting for nearly 50% of the total cases (Zimmermann & Hurrell, 2007). Due to blood loss during their menstrual cycles, women are 10 times more susceptible to developing IDA than men of the same age (Garzon et al., 2020). Anemia is more common in the elderly; current research indicates that 10% of people over the age of 65 are anemic, with IDA accounting again for half of these cases (Guralnik et al., 2004). IDA occurs due to chronic diseases (inflammation and chronic kidney disease) or nutritional deficiencies (Busti et al., 2014). IDA is present in two forms: functional and absolute. In absolute IDA, the amount of iron is insufficient to support the production of RBCs, whereas, in functional IDA, the amount of iron is sufficient, but the body fails to supply it to the bone marrow to produce RBCs (Busti et al., 2014; Camaschella & Girelli, 2020).

IDA arises from different physiological and pathological reasons, including inadequate iron uptake (deficient diet, inadequate iron absorption), an increased iron requirement for growth and/or pregnancy, blood loss due to several diseases, or different physiological reasons like menstrual cycles (Camaschella, 2015; Clark, 2008; Lopez et al., 2016; Pasricha et al., 2021). This disease can also be developed after treatment with several drugs like nonsteroidal anti-inflammatory drugs (NSAIDs), which are the most common medication used for treating pain, fever, and other inflammatory diseases (Wongrakpanich et al., 2018). NSAIDs include diclofenac, ibuprofen, ketoprofen, and naproxen, to name but a few (Ghlichloo & Gerriets, 2019). Finally, it should be noted that genetic disorders may also cause IDA (Camaschella, 2015). Development of IDA has three stages: storage iron deficiency (or decreased iron stores), iron deficient erythropoiesis (iron depletion in the bone marrow), and IDA (low Hb level and development of anemia) (Scott & Stockham, 2013). It should be noted that iron reserves are initially prioritized for increased erythropoiesis during blood loss (Naigamwalla et al., 2012). Erythropoiesis and the creation of other iron-containing proteins (such as myoglobin) are restricted once the body's iron reserves are depleted, which causes IDA (Feldman et al., 2000).

The main symptoms of IDA are fatigue, headaches, paleness, and cold intolerance (DeLoughery, 2017; Lopez et al., 2016). Sometimes symptoms like tachycardia, lack of endurance, pica (an eating disorder that includes eating nonfood items), and koilonychia, are also observed (DeLoughery, 2017; Gao & Monaghan, 2018). IDA has extensive negative consequences on immunity, including impaired B-cell and T-cell activity, as well as reduced phagocytosis and macrophage-killing ability (Scrimshaw & SanGiovanni, 1997). It is also linked to an increased risk of malaria and infection (Oppenheimer, 2001). IDA diagnosis entails determining the underlying condition or cause through a thorough review of the patient's medical history and physical examination. A thorough examination of the patient's medications, nutrition, concurrent medical conditions, fecal features, exposure to fleas and ticks, and careful questioning regarding potential sources of blood loss should be performed (Naigamwalla et al., 2012). Generally, diagnostic testing includes performing a complete blood count test, peripheral smear, reticulocyte count, and serum iron indices testing (Johnson-Wimbley & Graham, 2011). Hb determination has been the most extensively used screening tool for iron deficiency, however, it has limitations for the diagnosis of IDA when employed as the sole laboratory test due to its low specificity and sensitivity (Cook, 2005). For the determination of IDA, the central parameter is ferritin (Miller, 2013). In most cases, ferrous sulfate is used to treat this condition (DeLoughery, 2017). However, other iron supplements (iron dextran, iron gluconate, and iron sucrose) are also widely used for IDA management (Cook, 2005).

### 3.1.2 | ACD

ACD is the second most common anemia after IDA (Weiss & Goodnough, 2005). It occurs due to the inability of the bone marrow to produce enough RBCs (also called hypoproliferative anemia) even though there is enough reticuloendothelial iron available (Means RTMeans, 2003). Recent studies have shown that ACD is linked to obesity, aging, kidney failure, and chronic inflammatory diseases like cancer, chronic infection, and autoimmune diseases (Fraenkel, 2015). In patients who suffer from chronic inflammatory states like cancer and autoimmune diseases, ACD is also found (Weiss, 2009). For this reason, this disease is also referred to as anemia of inflammation (Fraenkel, 2015). As presented above, the level of hepcidin is controlled by a complex interaction between bone morphogenic proteins, its coreceptor hemojuvelin, proteases, and inflammatory cytokines, like IL-1 and IL-6 (Fraenkel, 2015; Lee et al., 2005). In the case of inflammation, IL-1 and IL-6 increase the production of hepcidin (Lee et al., 2005). Due to the increase of hepcidin levels in serum, macrophages engulf senescent and old erythrocytes and thus, acquire iron, but it is not released, which causes ACD (Agarwal & Prchal, 2009).

Measuring Hb levels is not enough for the correct diagnosis of ACD since the exclusion of other kinds of anemia (particularly IDA) is needed for the diagnosis of ACD (Wiciński et al., 2020). Some other

parameters like serum iron levels and transferrin saturation are key for distinguishing ACD from other anemias (in ACD iron levels and transferrin saturation are decreased whereas transferrin levels are increased in IDA but they remain almost normal or decreased in ACD) (J. Cullis, 2013; Wiciński et al., 2020). With regard to therapy, the main treatment of ACD evolves around eradicating the underneath disease that promotes its development (Fraenkel, 2015). If that is not possible, blood transfusions or iron supplements can be used to treat this disorder (Nemeth & Ganz, 2014). In this regard, treatment of the underlying inflammatory or malignant process associated with ACD will often result in an improvement in the degree of anemia; examples include the use of corticosteroids in polymyalgia rheumatica, tumor necrosis factor inhibitors in rheumatoid arthritis or inflammatory bowel disease as well as the use of antiretroviral medications in the treatment of human immunodeficiency virus infection (J. O. Cullis, 2011).

### 3.1.3 | Thalassemia

Thalassemia is a type of anemia caused by a genetic disorder, which reduces the adequate production of the  $\alpha$  or  $\beta$  chains of the Hb (Aydinok, 2012; Muncie HL & Campbell, 2009; Vij & Machado, 2010). This disease is prevalent in Indian, Mediterranean, South Chinese, Middle Eastern, and Southeast Asian descent (Li, 2017). As it is an autosomal recessive disorder, both parents must be affected by or be carriers of the gene to pass it to their offspring (Bajwa & Basit, 2019). Thalassemia can be divided into two major groups:  $\alpha$ -thalassemia, which occurs when there is a decrease in the production of the  $\alpha$ -globin chain, thus leading to an increase in the production of the  $\beta$ -globin chain in Hb, and  $\beta$ -thalassemia, in which the production of the  $\beta$ -globin chain is decreased and leads to the increase of the  $\alpha$ -globin chain in the molecule (Helmi et al., 2017; Muncie HL & Campbell, 2009; Rund & Rachmilewitz, 2001). Mutation or deletion of one or more of the genes responsible to produce  $\alpha$ -globin chains (situated in chromosome 16) causes  $\alpha$ -thalassemia (Bajwa & Basit, 2019; Li, 2017; Muncie HL & Campbell, 2009). The four clinically recognized forms of  $\alpha$ -thalassemia are: silent carrier,  $\alpha$ -thalassemia trait, Hb H (HbH) disease, and hydrops fetalis syndrome (Marengo-Rowe, 2007).  $\alpha$ -thalassemia silent carrier status is caused by a single gene loss and is asymptomatic with normal hematologic findings (Muncie HL & Campbell, 2009).  $\alpha$ -thalassemia trait is associated with microcytosis and typically, no anemia is presented by this two-gene deletion (Hartevelde & Higgs, 2010). However, the three-gene loss leads to considerable HbH synthesis, which contains four  $\beta$ -chains (Songdej & Fucharoen, 2022). HbH can cause chronic hypochromic microcytic anemia and hemolytic anemia (Harewood & Azevedo, 2017). Hb Barts, which has four gamma chains, is significantly produced as a result of the four-gene deletion. Usually,  $\alpha$ -thalassemia major with Hb Barts causes fatal hydrops fetalis (Chui et al., 1998). On the other hand, mutation of the  $\beta$ -globin gene cluster in any of its 200 regions (since deletion of the gene is rare) in chromosome 11 results in the development of  $\beta$ -thalassemia

(Aydinok, 2012; Bajwa & Basit, 2019; Galanello & Origa, 2010; Marengo-Rowe, 2007; Muncie HL & Campbell, 2009). The synthesis of  $\beta$ -chains is completely absent in  $\beta^0$ -thalassemia patients; in  $\beta^+$ -thalassemia there is a partial shortage in the formation of  $\beta$ -chains (Marengo-Rowe, 2007).

The symptoms of thalassemia are dependent on its severity and type. Common symptoms include: pale skin, deformed facial or skeletal bones, and arrhythmias (Bajwa & Basit, 2019). Symptoms may be absent in silent carriers of  $\alpha$ -thalassemia (Shafique et al., 2021). For diagnosis, measurements of the mean corpuscular volume (MCV) of individual RBCs are used to differentiate between IDA and thalassemia (Galanello & Origa, 2010; Muncie HL & Campbell, 2009). More specifically, the Mentzer index, which is the MCV divided by the RBC count, is used to differentiate thalassemia from iron deficiency (indexes with a value of  $<13$  serve to diagnose the person with thalassemia, whereas a value of  $>13$  is found in patients with iron deficiency) (Siswandari et al., 2019). As for the therapy, patients with thalassemia traits do not need any treatment (Ali et al., 2021). On the other hand, supplements of folic acid (2–5 mg/day) are prescribed to HbH patients (Vichinsky, 2012). However, during times of potential oxidative stress, such as infections or the use of oxidative drugs, blood counts should be monitored and transfusional intervention may be necessary (Harewood & Azevedo, 2017). Also, for mild thalassemia, occasional blood transfusions may be needed, whereas for moderate to severe thalassemia, frequent blood transfusions, the use of chelation therapy, stem cell transplants, and even gene therapy may be required (Bajwa & Basit, 2019).

### 3.1.4 | Sickle cell anemia (SCA)

SCA is an autosomal recessive disorder caused by a mutation in the  $\beta$ -globin gene of the Hb. In this mutation, the substitution of glutamic acid by valine occurs, resulting in the production of sickle Hb (HbS) molecules, which interact with adjacent HbS molecules under deoxygenation conditions and create HbS polymers (M. R. H. Weigand et al., 2021). These aggregates form lengthy chains, distorting the RBC into a sickled shape and impeding the cell's passage through blood vessels (Lonergan et al., 2001). Furthermore, the malformed RBCs tend to attach to the endothelium, which results in the reduction of the cell deformability and hindrance in blood vessels, promoting the development of excruciatingly painful vaso-occlusive crisis (VOC) (Darbari et al., 2020; Eaton et al., 2006; Jang et al., 2021). Polymerization also increases the permeability of the cell's membrane for cations (sodium, potassium, magnesium, and calcium) in a nonselective manner. When these cations reach the RBCs, numerous cell membrane transport processes are triggered, with the most significant consequence being the water outflow from the RBCs, which in turn, increases the density of the cells (Lonergan et al., 2001).

Apart from VOC, SCA patients suffer from invasive infections, acute chest syndrome, strokes, and chronic pulmonary hypertension (Mehta et al., 2006; Shah et al., 2019). The median life expectancy of



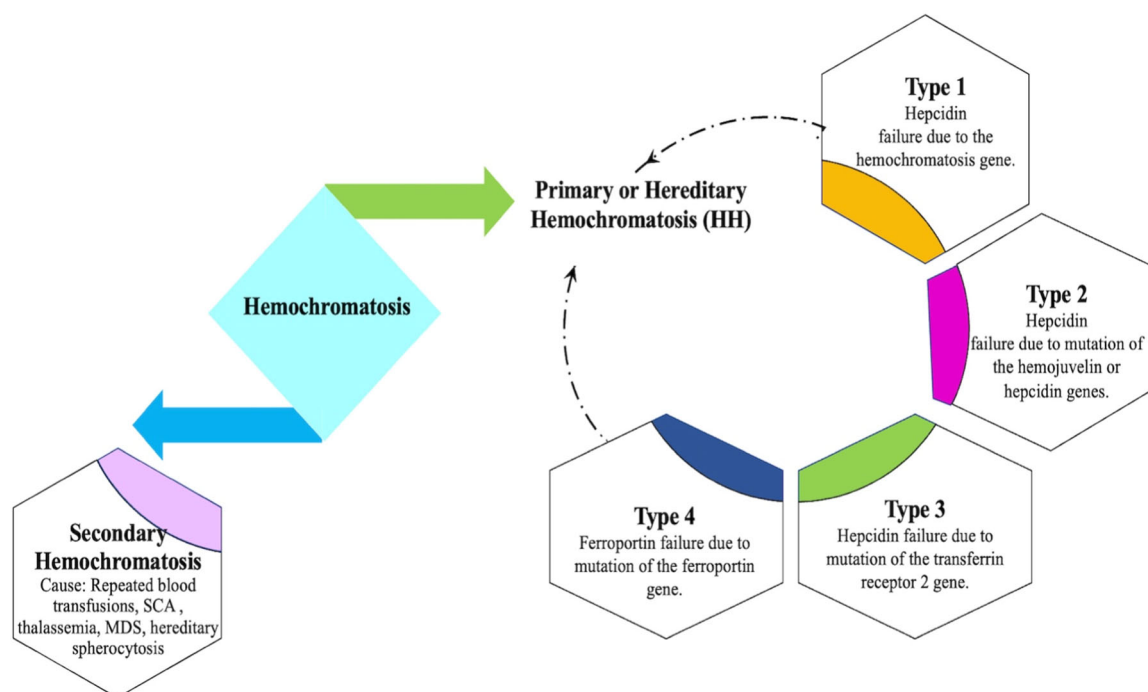
SCA patients is between 42 and 47 years (Hematology, 2016). This mutation is common in sub-Saharan Africa and every year almost 300,000 individuals are born with the disease (Mburu & Odame, 2019). The inheritance of both HbA (normal Hb) and HbS (or HbAS) corresponds to sickle cell trait; strictly not a form of SCA but that may be associated with adverse health outcomes (M. Weigand, Pastora, Palmer, et al., 2022; M. Weigand, Gomez-Pastora, Strayer, et al., 2022). Sickle cell trait affects between 1 and 3 million Americans, 8%–10% of African Americans, and more than 100 million people worldwide (Hematology). It should be noted that there is no recommended approach for diagnosing SCD (Smith & Kinney, 1997). Nevertheless, the Agency for Health Care Policy and Research has proposed combining three newborn screening methods: Hb electrophoresis, isoelectric focusing, and high-performance liquid chromatography (HPLC) (Koch et al., 2000). Apart from those, other diagnostic techniques have been developed to determine SCA (Ilyas et al., 2020; Ware et al., 2017). Moreover, microfluidic systems and other point-of-care (POC) devices have been successfully tested for the diagnosis of SCA (Hasan, 2017; Knowlton et al., 2015). Modern treatment options for SCA emphasize optimum supportive care, such as blood transfusions and pain relievers (Tisdale et al., 2020). Drugs like hydroxyurea or voxelotor are widely used for treating SCA patients (Eaton & Bunn, 2017; Platt et al., 1984). Other treatments for SCA include hydroxycarbamide, L-glutamine, and bone marrow transplantation (Agrawal et al., 2014; Inusa et al., 2019; Lonergan et al., 2001). Significant improvements in SCA understanding have enabled the development of curative therapeutics via allogeneic stem cell transplantation, with the prospect of gene therapy-based treatments in the future (Tisdale et al., 2020).

### 3.1.5 | Vitamin deficiency anemia

Vitamin deficiency or nutritional anemia is considered the most common nutritional disorder in the world, mainly prevalent in developing countries (Ahmed, 2000; Hercberg & Rouaud, 1981). To ensure the normal production of blood cells, some vitamins like vitamin A, folic acid, vitamin B12, vitamin B6, and riboflavin (vitamin B2) play a vital role, where vitamin C and E work as antioxidants by diminishing the presence of free radicals (Fishman et al., 2000). Vitamin A appears to be involved in the pathogenesis of anemia through diverse biological mechanisms, but these are not fully understood yet (Semba & Bloem, 2002). The deficiency of folate and vitamin B12 impairs DNA synthesis and causes megaloblastic anemia (Ayodele, 2018). On the other hand, riboflavin deficiency leads to anemia by impairing iron absorption and iron metabolism (Mahabadi et al., 2022; Meier et al., 1981). Finally, vitamin B6 is used for treating sideroblastic anemia but deficiency of this vitamin is rare (Fishman et al., 2000; Meier et al., 1981).

## 3.2 | Hemochromatosis

Hemochromatosis, which can be resulted from both acquired and genetic conditions, is one of the most common hematologic disorders, characterized by iron overload (Yun & Vincelette, 2015). It is also known as 'bronze diabetes' as this disease changes the color of the skin and creates pancreas-related disorders (Porter & Rawla, 2021). The different types of hemochromatosis are shown in Figure 6. Hemochromatosis can be divided into two types: primary or hereditary hemochromatosis (HH) and secondary hemochromatosis (Porter & Rawla, 2021; Siddique &



**FIGURE 6** Different types of hemochromatosis and their mechanisms.

Kowdley, 2012). It is most prevalent in Caucasian descents who are homozygote or compound heterozygote for genes on chromosome 6p (Barton et al., 1998; Blachier et al., 2013; Zoller & Henninger, 2016). In Celtic and Nordic countries, approximately 1 in 220 people are affected by HH (Bacon et al., 2011). Due to blood loss in menstrual cycles, women are less affected by HH and the ratio between men and women is 3:1 (Porter & Rawla, 2021).

Generally, HH is a genetic condition (Brissot et al., 2008). If the iron levels in plasma are more than enough to support erythropoiesis, NTBI starts accumulating in the liver, heart, and endocrine tissue and eventually causes HH (Hider & Kong, 2013). The extra NTBI can also produce cytotoxic hydroxyl and lipid radicals in the liver, heart, and pancreas and lead to the failure of these organs (Yun & Vincelette, 2015). Recent research has shown that the monocytes of HH patients release twice lower molecular weight iron (LMW-Fe) than what monocytes from healthy individuals release (Kim, Gómez-Pastora, Weigand, et al., 2019). This LMW-Fe binds easily to plasma transferrin and contributes to the high NTBI observed in these patients (Moura et al., 1998). The symptoms of HH include weakness, fatigue, weight loss, abdominal pain, diabetes, and loss of libido (Bacon et al., 2011).

HH can be divided into four subgroups: type 1 or HFE-related hemochromatosis, type 2 or juvenile hemochromatosis, type 3 hemochromatosis, and type 4 hemochromatosis, or ferroportin disease (Bacon et al., 2011; Brissot et al., 2008). Major mutation of the HFE gene causes type 1 or HFE-related hemochromatosis (Milman et al., 2019). This mutation leads to a lower hepcidin expression in these patients, which promotes higher intestinal iron absorption (Bridle et al., 2003). Hepcidin deficiency or hepcidin resistance also results in impaired control of iron storage in macrophages and increased iron release into the circulation (Fillet et al., 1989). Consequently, and despite the fact that iron excess is present in other tissues, individuals with hemochromatosis have inadequate levels of iron in their macrophages (McLaren, 1989). Type 2 or juvenile hemochromatosis is a rare disease that affects mostly young individuals (Brissot et al., 2008) and is caused by hepcidin failure due to the mutation of hemojuvelin (Lu et al., 2004) or hepcidin (Roetto et al., 2003). On the other hand, type 3 hemochromatosis is an autosomal recessive disease that occurs due to the mutation of the Tfr-2 gene, which is located on chromosome 7 (Roetto et al., 2001). When type 3 HH results in an iron overload comparable to HFE-related hemochromatosis, it can cause aberrant liver function, diabetes, hypogonadism, cardiomyopathy, and arthritis (Lima Santos et al., 2012). Type 4 hemochromatosis results from the mutation of the ferroportin (SLC40A1) gene (Pietrangelo, 2004; Wallace & Subramaniam, 2007). This type of hemochromatosis is more frequent than types 2 and 3 and it has been reported in a wide range of individuals (Bardou-Jacquet & Brissot, 2014); since it affects ferroportin, this disorder causes loss of the iron export function (De Domenico et al., 2006; Drakesmith & Prentice, 2012). As presented above, two types of mechanisms are involved in HH: hepcidin failure and ferroportin failure (Brissot et al., 2008). Hepcidin failure increases NTBI (Hider, 2002) and this is the responsible mechanism behind types 1, 2, and 3 hemochromatoses (Brissot et al., 2008). Type 4 arises from the mutation of the ferroportin gene, which leads to ferroportin failure (Brissot et al., 2008; Hider & Kong, 2013). On the other

hand, secondary hemochromatosis arises from repeated transfusions of blood, and may be caused by diseases like thalassemia, myelodysplastic syndromes, SCA, and hereditary spherocytosis (Gattermann, 2009; Hider & Kong, 2013; Porter & Rawla, 2021).

High serum ferritin and transferrin saturation are observed in patients with hemochromatosis (in general, serum ferritin levels above 300 ng/ml in men and 200 ng/ml in women are found (Elsayed et al., 2016)). Nevertheless, other possible disorders such as acute or chronic inflammatory conditions, liver illness, infection, and secondary iron overload, must be taken into account while evaluating hemochromatosis (Murphree et al., 2020). Patients with continuously high ferritin and transferrin saturation levels who do not present any other evident iron overload reason need to be considered for HFE mutation testing. In the absence of tests showing excessive iron levels, it is prudent for individuals with a known family history of hemochromatosis to undertake HFE mutation testing, especially if both parents are Caucasian due to the greater occurrence of HFE mutations in people of European heritage (Bardou-Jacquet & Brissot, 2014). Patients with a recent hemochromatosis diagnosis should be examined for typical signs of organ damage, paying particular attention to the liver, heart, pancreas, anterior pituitary, joints, and skin (Murphree et al., 2020). The most common and general treatment for this condition includes therapeutic phlebotomy (Witte et al., 1996). Clinical evidence shows that iron removal before the development of cirrhosis and diabetes is related to a decreased morbidity and mortality (Niederau et al., 1996), and that some aspects such as hepatic fibrosis, may be alleviated by a phlebotomy treatment (Powell et al., 2006). Nevertheless, if a patient does not have access to a superior vein or if venesection is contraindicated, iron chelators like deferasirox may be recommended for early iron removal (Adams et al., 2000; Phatak et al., 2010).

## 4 | MAGNETOPHORESIS AS A POTENTIAL SEPARATION TECHNIQUE FOR THE DIAGNOSIS AND TREATMENT OF IRON-RELATED DISORDERS

### 4.1 | Fundamentals of blood cell magnetophoresis

Magnetophoresis refers to the movement of a magnetic particle (and/or cell or biological matter) in a viscous medium under influence of inhomogeneous magnetic fields (Robert et al., 2011; Watarai & Namba, 2002; Zborowski et al., 2002). Publications on magnetophoresis have increased in recent years due to its versatile application in biomedical research, water treatment, diagnosis of diseases, and therapy (Gómez Pastora et al., 2016; Gómez-Pastora et al., 2020; Lenshof & Laurell, 2010; Wu et al., 2022; Zborowski & Chalmers, 1999). The primary advantage of magnetophoresis is that it enables the specific manipulation of thousands of particles and cells efficiently over a short period of time (e.g., magnetophoretic-based devices have been able to separate continuously RBCs from WBCs at relatively high volumetric flowrates, comparable to other devices used for magnetic particle

separation at the microscale) (K. H. Han & Frazier, 2006; Hejazian et al., 2015; Whitesides, 2006). Figure 7 presents the acting forces on blood cells in a magnetophoretic device. The motion of particles and cells in a typical magnetophoretic device depends on several competing forces, such as the drag force ( $\vec{F}_D$ ), magnetophoretic force ( $\vec{F}_M$ ), buoyancy force ( $\vec{F}_B$ ), gravitational force ( $\vec{F}_G$ ), and lift force ( $\vec{F}_L$ ). In most cases, for a shallow channel and a particle/cell suspended in an incompressible fluid,  $\vec{F}_B$ ,  $\vec{F}_G$ , and  $\vec{F}_L$ , can be neglected (Alnaimat et al., 2018; Pamme & Manz, 2004; Gómez-Pastora et al.).

Under this scenario, the particle/cell trajectory can be computed according to Newton's second law (González Fernández et al., 2020; González-Fernández et al., 2021; X. Han et al., 2015):

$$m_p \frac{d\vec{v}_p}{dt} = \vec{F}_D + \vec{F}_M \quad (1)$$

where,  $m_p$  represents the mass of the particle (or blood cell) and  $\vec{v}_p$  its velocity. Applying Stoke's law,  $\vec{F}_D$  can be obtained as (Furlani, 2007; Gijs et al., 2010):

$$\vec{F}_D = 6\pi r \mu_f (\vec{v}_f - \vec{v}_p) f_D \quad (2)$$

where  $r$  represents the hydraulic diameter of the particle/cell,  $\mu_f$  is the viscosity of the fluid,  $\vec{v}_f$  is the velocity of the fluid, and  $f_D$  is the drag coefficient, which can be estimated as:

$$f_D = \left\{ 1 - \frac{9}{16} \left( \frac{r}{r+z} \right) + \frac{1}{8} \left( \frac{r}{r+z} \right)^3 - \frac{45}{256} \left( \frac{r}{r+z} \right)^4 - \frac{1}{16} \left( \frac{r}{r+z} \right)^5 \right\}^{-1} \quad (3)$$

where,  $z$  represents the distance of the particle to the solid wall of the channel/device. For small distances (which is generally the case for cell separation in small devices), one may consider  $z$  negligible, in

which case ( $z = 0$ ),  $f_D = 3$  (Gijs et al., 2010). Following,  $\vec{F}_M$  can be calculated as follows (Amiri Roodan et al., 2020; Hejazian et al., 2015; Munaz et al., 2018; Shiriny & Bayareh, 2020; Tarn & Pamme, 2017):

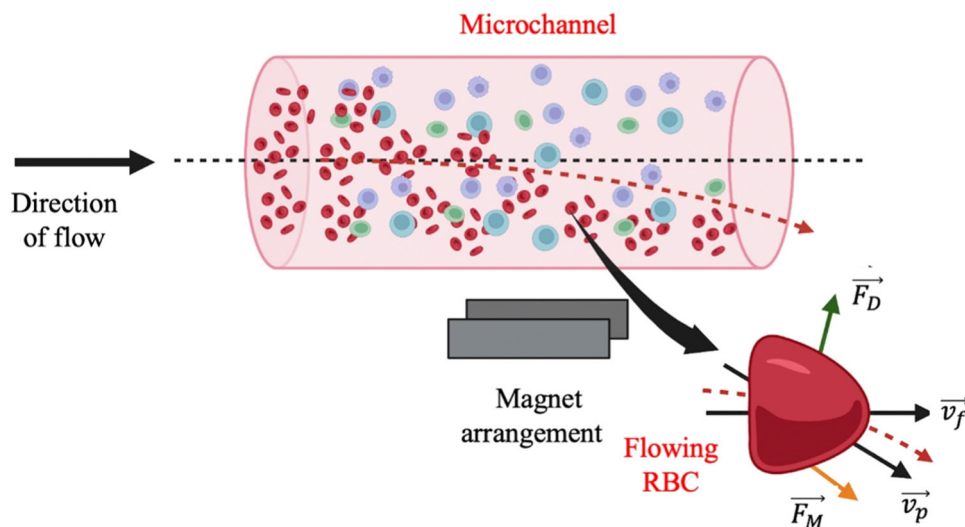
$$\vec{F}_M = \frac{V_p}{\mu_0} (\chi_p - \chi_f) (\vec{B} \cdot \nabla) \vec{B} \quad (4)$$

where,  $V_p$  is the volume of the cell,  $\chi_p$  and  $\chi_f$  are the dimensionless magnetic susceptibilities of the particle/cell and fluid, respectively, and  $\mu_0$  is the magnetic permeability in vacuum ( $\mu_0 = 4\pi \times 10^{-7}$  H/m).  $\vec{B}$  and  $\nabla \vec{B}$  are the magnetic flux density and its gradient. The magnetic susceptibility ( $\chi$ ) value is generally employed to classify materials into ferromagnetic ( $\chi \gg 0$ ), paramagnetic ( $\chi > 0$ ), and diamagnetic ( $\chi < 0$ ) (Munaz et al., 2018). In whole blood, most WBCs show diamagnetism, whereas RBCs show diamagnetism or paramagnetism depending on their oxygenated state (Furlani, 2007). As oxyhemoglobin has no unpaired electrons, it shows zero magnetic moment and diamagnetic behavior, whereas deoxyhemoglobin is paramagnetic since it has four unpaired electrons (Pauling & Coryell, 1936a). On the other hand, methemoglobin contains five unpaired electrons in its structure and shows paramagnetism (Zborowski et al., 2003). Since monocytes acquire iron from RBCs, some cells have magnetic properties. More specifically, recent research has shown that a subset of monocytes has paramagnetic properties (Gómez-Pastora et al., 2021).

## 4.2 | Applications of blood cell magnetophoresis

### 4.2.1 | Diagnosis of anemia

For diagnosing anemia, determination of the RBC count, hematocrit (Hct), and Hb is performed (Gómez-Pastora, Weigand, et al., 2022).

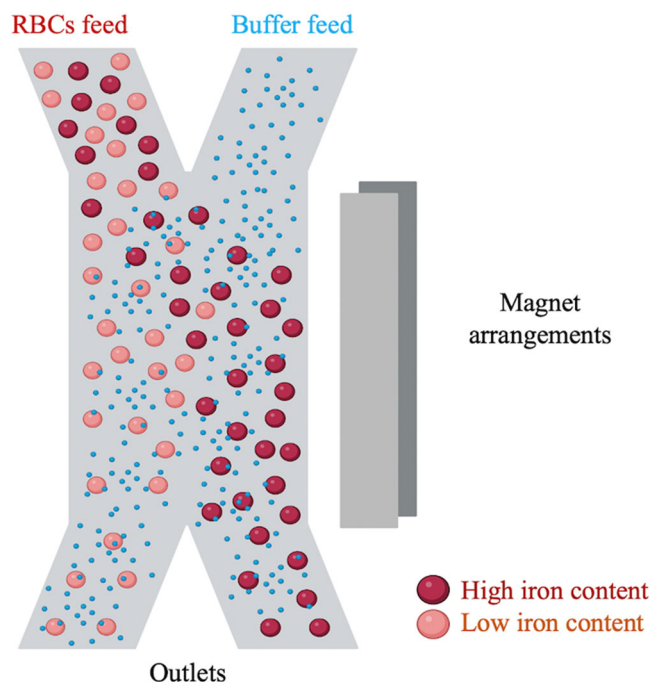


**FIGURE 7** The acting forces on a cell in a magnetophoretic system. Adapted and slightly edited from Gomez-Pastora et al. (Gómez-Pastora et al., 2017).

Also, Wintrobe's parameters such as MCV, mean corpuscular Hb (MCH), and MCH concentration (MCHC) of RBCs can be helpful to find the etiology of the anemia (Sarma, 1990). Some modern blood analyzers use flow cytometry, spectrum absorption, and/or chemical reaction to provide new useful indices like reticulocyte counts, and hypochromic RBC counts (Schaefer & Schaefer, 1999). Although these instruments provide accurate and reproducible data, they are very expensive (\$75,000–\$200,000) (Sullivan, 2006), present issues in portability, and require trained technicians and costly reagents to operate (Heikali & Di, 2010). According to Pauling and Coryell's pioneering work on the magnetism of Hb, different states of Hb show different magnetic moments (Pauling & Coryell, 1936a, 1936b), as has been presented above. Under this scenario, the use of magnetophoresis can be a cheaper solution to diagnose anemias since the introduction of powerful and low-cost neodymium magnets and modern computer imaging technologies enable the development of instruments that can determine the magnetic susceptibility of blood cells, which are related to their Hb and iron content (Chalmers et al., 1999, 2017). For example, recent works have suggested the use of a permanent magnet-based microsystem referred to as cell tracking velocimetry (CTV) (Chalmers et al., 1999; Kim, Gómez-Pastora, Gilbert, et al., 2019; Kim, Gómez-Pastora, Weigand, et al., 2019; R. H. Weigand et al., 2021) as a portable and inexpensive hematology analyzer able to measure traditional RBC indices and novel indices such as the percentage of hypochromic RBCs and the red cell distribution width (Gómez-Pastora, Weigand, et al., 2022).

#### 4.2.2 | Separation of healthy and unhealthy cells based on iron content (fractionation)

In the case of surgery, trauma resuscitation, treatment of patients with oncologic diseases, organ transplantation, severe blood loss, and acute anemia, blood transfusions can be considered a lifesaving treatment (Kim, Gómez-Pastora, Gilbert, et al., 2019; W. B. Ross & Yap, 1990). After transfusion, 25% of the transfused RBCs are removed by the spleen even though the *ex-vivo* damage of the exogenous RBCs is minimal (M. Weigand Pastora, Palmer, et al., 2022; M. Weigand, Gomez-Pastora, Strayer, et al., 2022). Receiving multiple transfusions is associated with several side effects. Due to RBC units shortage problems, some hospitals diluted RBC units during the coronavirus disease 2019 (COVID-19) pandemic (Jacobs & Booth, 2022), which may have alleviated the problems associated with receiving repeated blood transfusions like alloimmunization, iron overload, and delayed hemolytic reactions (Ware et al., 2017). Magnetophoresis can also be a solution to fractionate *ex-vivo*, stored RBCs, to separate healthy, iron-rich, RBCs from damaged or senescent RBCs, and thus, provide effective and high-quality cells for transfusion (Gómez-Pastora et al., 2018; McCloskey et al., 2003; Munaz et al., 2018). A possible setup for RBC separation based on iron content is shown in Figure 8. A recent study evaluated the



**FIGURE 8** Schematics of blood cell fractionation based on iron levels.

possibility of using magnetophoresis (by using a neodymium-iron-boron [NdFeB] permanent magnet in a pressure-driven flow system) to fractionate RBCs based on their iron content (M. Weigand, Pastora, Palmer, et al., 2022; M. Weigand, Gomez-Pastora, Strayer, et al., 2022).

#### 4.2.3 | Sick cell disease diagnosis and treatment

Diagnosis of SCA requires HPLC, Hb electrophoresis, and/or isoelectric focusing, which are expensive analyses and require trained technicians (Yusuf et al., 2011). Since SCA is the most frequent Hb abnormality in sub-Saharan Africa, where the health care system is inaccessible to the majority of the population (Ngolet et al., 2016), a low-cost, compact, and user-friendly diagnostic device is needed (Knowlton et al., 2015). Since one of the main characteristics of SCA is the presence of high-density RBCs in the bloodstream (Fabry et al., 1984), density-based diagnosis is a cheap alternative to the existing ones (Kumar, Patton, et al., 2014). In this regard, magnetic levitation (MagLev) can be a promising alternative because of its low cost. This technology can distinguish between two diamagnetic objects whose densities differ by  $0.0002 \text{ g/cm}^3$  (Abrahamsson et al., 2020; Ge & Whitesides, 2018; Mirica et al., 2009). MagLev is performed by suspending the material in a medium; then, a combination of magnetic and gravity forces separates the objects according to their density (Ge et al., 2020). A recent study suggested the potential use of a cheap, portable diagnosis system that incorporates a three-dimensional printed MagLev platform with a cellphone and induces cell dehydration by sodium metabisulfite



(Knowlton et al., 2015). Other studies have also employed MagLev-based systems as POC diagnostic devices for SCA (Kumar, Chunda-Liyoka, et al., 2014; Song et al., 2014).

Furthermore, for SCA, regular blood transfusions are recommended in the case of acute anemia, splenic sequestration, reticulocytopenia, accelerated hemolysis, stroke, and acute chest syndrome (Telen, 2001). In the case of VOC, many patients need blood transfusion therapy to reduce the concentration of HbS (Boma Muteb et al., 2017). During the procedure, the blood of the patient is discarded through an apheresis device, and replaced by healthy RBCs (Howard, 2016; Quirolo et al., 2015). However, only a small portion of the RBCs in the SCD patients are polymerized (M. Weigand, Pastora, Palmer, et al., 2022; M. Weigand, Gomez-Pastora, Strayer, et al., 2022). In this regard, magnetophoresis is a potential solution to separate the patient's sickle RBCs from the healthy RBCs due to their different oxygen affinities, which affect the RBC magnetic properties (Eldeniz et al., 2021; M. Weigand, Pastora, Palmer, et al., 2022; M. Weigand, Gomez-Pastora, Strayer, et al., 2022). A recent study evaluated the potential of magnetophoresis using a NdFeB permanent magnet in a pressure-driven flow system to separate healthy RBCs from sickle RBCs and further research in this area may yield future therapeutic interventions (M. Weigand, Gomez-Pastora, Palmer, et al., 2022; M. Weigand, Gomez-Pastora, Strayer, et al., 2022).

## 5 | CONCLUSIONS

Iron in RBCs plays the main role of transporting oxygen and iron in monocytes/macrophages results from phagocytosing senescent RBCs, recycling it back to the bone marrow. Iron not only helps lymphocytes in performing their functions but also controls cytokines derived from T cells and macrophages. To perform these roles properly, iron content must be strictly regulated in the human body. Imbalances in iron physiology may lead to a wide spectrum of diseases ranging from anemia to hemochromatosis, some of which can be fatal. In this study, we have reviewed explicitly the different roles of iron in blood cells and have introduced several iron-related diseases such as IDA, SCA, thalassemia, ACD, and the different forms of hemochromatosis. Even though several techniques have been developed for the diagnosis and treatment of iron-related disorders, in some cases, they are not cost-effective, portable, or require trained technicians. Since some of the iron-related diseases affect the majority of the population of developing nations where health care resources are limited, combined with the current worldwide limited access to proper health care and treatment, magnetophoretic-based technologies emerge as a cheap, viable, and portable solution for the diagnosis of iron-related diseases, thus becoming a prominent research field. In this review, we have discussed several magnetophoretic devices that may offer a solution to these issues. For example, devices like the CTV have shown a new path in the diagnosis of iron-related diseases, like IDA. Other permanent magnet-based devices allow the fractionation of blood cells based

on iron content, which is a promising technology for the treatment of SCA. Finally, we introduce MagLev-based devices, which can be used as POC diagnostic devices for disorders like SCA.

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## DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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## REFERENCES

- Abrahamsson, C. K., Nagarkar, A., Fink, M. J., Preston, D. J., Ge, S., Bozenko, J. S., & Whitesides, G. M. (2020). Analysis of powders containing illicit drugs using magnetic levitation. *Angewandte Chemie International Edition*, 59(2), 874–881.
- Adams, P., Brissot, P., & Powell, L. W. (2000). EASL international consensus conference on haemochromatosis: Part II. *Expert Document. Journal of hepatology*, 33(3), 487–496.
- Agarwal, N., & Prchal, J. T. (2009). Anemia of chronic disease (anemia of inflammation). *Acta Haematologica*, 122(2–3), 103–108.
- Agrawal, R. K., Patel, R. K., Shah, V., Nainiwal, L., & Trivedi, B. (2014). Hydroxyurea in sickle cell disease: Drug review. *Indian Journal of Hematology and Blood Transfusion*, 30(2), 91–96.
- Ahmed, F. (2000). Anaemia in Bangladesh: A review of prevalence and aetiology. *Public Health Nutrition*, 3(4), 385–393.
- Alfadda, A. A., & Sallam, R. M. (2012). Reactive oxygen species in health and disease. *Journal of Biomedicine and Biotechnology*, 2012, 1–14.
- Ali, S., Mumtaz, S., Shakir, H. A., Khan, M., Tahir, H. M., Mumtaz, S., Mughal, T. A., Hassan, A., Kazmi, S., Irfan, M., & Khan, M. A. (2021). Current status of beta-thalassemia and its treatment strategies. *Molecular Genetics & Genomic Medicine*, 9(12), 1788.
- Alnaimat, F., Dagher, S., Mathew, B., Hilal-Alnqbi, A., & Khashan, S. (2018). Microfluidics based magnetophoresis: A review. *The Chemical Record*, 18(11), 1596–1612.
- Althuwaiqeb, S. A., & Bordon, B. (2021). *Histology, B Cell Lymphocyte*, in *StatPearls [Internet]*. StatPearls Publishing.
- Amiri Roodan, V., Gómez-Pastora, J., Karampelas, I. H., González-Fernández, C., Bringas, E., Ortiz, I., Chalmers, J. J., Furlani, E. P., & Swihart, M. T. (2020). Formation and manipulation of ferrofluid droplets with magnetic fields in a microdevice: A numerical parametric study. *Soft Matter*, 16(41), 9506–9518.
- Ashton, N. (2007). Physiology of red and white blood cells. *Anaesthesia & Intensive Care Medicine*, 8(5), 203–208.
- Aydinok, Y. (2012). Thalassemia. *Hematology*, 17(sup1), s28–s31.
- Ayodele, O. J. (2018). Megaloblastic anemia. *Current Topics in Anemia. InTec. Nigeria*, 2, 29–44.
- Bacon, B. R., Adams, P. C., Kowdley, K. V., Powell, L. W., & Tavill, A. S. (2011). Diagnosis and management of hemochromatosis: 2011 practice guideline by the American association for the study of liver diseases. *Hepatology*, 54(1), 328–343.
- Bajwa, H., & Basit, H. (2019). *Thalassemia*.
- Barbusiński, K. (2009). Fenton reaction-controversy concerning the chemistry. *Ecological Chemistry and Engineering. S*, 16(3), 347–358.
- Bardaweel, S. K., Gul, M., Alzweiri, M., Ishaqat, A., ALSalamat, H. A., & Bashatwah, R. M. (2018). Reactive oxygen species: The dual role in physiological and pathological conditions of the human body. *The Eurasian Journal of Medicine*, 50(3), 193–201.

- Bardou-Jacquet, E., & Brissot, P. (2014). Diagnostic evaluation of hereditary hemochromatosis (HFE and non-HFE). *Hematology/Oncology Clinics of North America*, 28(4), 625–635.
- Barton, J. C., et al. (1998). Management of hemochromatosis. *Annals of Internal Medicine*, 129(11\_Part\_2), 932–939.
- Bayr, H. (2005). Reactive oxygen species. *Critical Care Medicine*, 33(12), S498–S501.
- Bessis, M., & Weed, R. I. (1973). The structure of normal and pathologic erythrocytes. *Advances in Biological and Medical Physics*, 14, 35–91.
- Blachier, M., Leleu, H., Peck-Radosavljevic, M., Valla, D. C., & Roudot-Thoraval, F. (2013). The burden of liver disease in Europe: A review of available epidemiological data. *Journal of Hepatology*, 58(3), 593–608.
- Blumenreich, M. S. (1990). *The white blood cell and differential count. Clinical Methods: The History, Physical, and Laboratory Examinations. 3rd edition.*
- Boma Muteb, P., Kaluila Mamba, J. F. J., Muhau Pfula, P., Bilo, V., Panda Mulefu, J. D., & Diallo, D. A. (2017). Effectiveness, safety, and cost of partial exchange transfusions in patients with sickle-cell anemia at a sickle cell disease center in sub-Saharan Africa. *Médecine et Santé Tropicales*, 27(4), 387–391.
- Bonaccorsi-Riani, E., Danger, R., Lozano, J. J., Martinez-Picola, M., Kodela, E., Mas-Malavila, R., Bruguera, M., Collins, H. L., Hider, R. C., Martinez-Llordella, M., & Sanchez-Fueyo, A. (2015). Iron deficiency impairs intra-hepatic lymphocyte mediated immune response. *PLoS One*, 10(8), e0136106.
- Bridle, K. R., Frazer, D. M., Wilkins, S. J., Dixon, J. L., Purdie, D. M., Crawford, D. H., Subramaniam, V. N., Powell, L. W., Anderson, G. J., & Ramm, G. A. (2003). Disrupted hepcidin regulation in HFE-associated haemochromatosis and the liver as a regulator of body iron homeostasis. *The Lancet*, 361(9358), 669–673.
- Brissot, P., Troade, M. B., Bardou-Jacquet, E., Lan, C. L., Jouanolle, A. M., Deugnier, Y., & Lortal, O. (2008). Current approach to hemochromatosis. *Blood Reviews*, 22(4), 195–210.
- Busti, F., Campostrini, N., Martinelli, N., & Girelli, D. (2014). Iron deficiency in the elderly population, revisited in the hepcidin era. *Frontiers in Pharmacology*, 5, 83.
- Bystrom, L. M., Guzman, M. L., & Rivella, S. (2014). Iron and reactive oxygen species: friends or foes of cancer cells? *Antioxidants & Redox Signaling*, 20(12), 1917–1924.
- Camaschella, C. (2015). Iron-deficiency anemia. *New England Journal of Medicine*, 372(19), 1832–1843.
- Camaschella, C. (2019). Iron deficiency. *Blood*, 133(1), 30–39.
- Camaschella, C., & Girelli, D. (2020). The changing landscape of iron deficiency. *Molecular Aspects of Medicine*, 75, 100861.
- Camaschella, C., Nai, A., & Silvestri, L. (2020). Iron metabolism and iron disorders revisited in the hepcidin era. *Haematologica*, 105(2), 260–272.
- Cappellini, M. D., Musallam, K. M., & Taher, A. T. (2020). Iron deficiency anaemia revisited. *Journal of Internal Medicine*, 287(2), 153–170.
- Chacon, A. H., Morrison, B., & Hu, S. (2013). Acquired hemochromatosis with pronounced pigment deposition of the upper eyelids. *The Journal of Clinical and Aesthetic Dermatology*, 6(10), 44–46.
- Chalmers, J. J., Haam, S., Zhao, Y., McCloskey, K., Moore, L., Zborowski, M., & Williams, P. S. (1999). Quantification of cellular properties from external fields and resulting induced velocity: Magnetic susceptibility. *Biotechnology and Bioengineering*, 64(5), 519–526.
- Chalmers, J. J., Jin, X., Palmer, A. F., Yazer, M. H., Moore, L., Amaya, P., Park, K., Pan, X., & Zborowski, M. (2017). Femtogram resolution of iron content on a per cell basis: Ex vivo storage of human red blood cells leads to loss of hemoglobin. *Analytical Chemistry*, 89(6), 3702–3709.
- Chaparro, C. M., & Suchdev, P. S. (2019). Anemia epidemiology, pathophysiology, and etiology in low-and middle-income countries. *Annals of the New York Academy of Sciences*, 1450(1), 15–31.
- Chui, D. H. K., Waye, J. S., Chui, D., & Waye, J. S. (1998). Hydrops fetalis caused by  $\alpha$ -thalassemia: An emerging health care problem. *Blood*, 91(7), 2213–2222.
- Clark, S. F. (2008). Iron deficiency anemia. *Nutrition in Clinical Practice*, 23(2), 128–141.
- Cook, J. D. (2005). Diagnosis and management of iron-deficiency anaemia. *Best Practice & Research, Clinical Haematology*, 18(2), 319–332.
- Cronin, S. J. F., Woolf, C. J., Weiss, G., & Penninger, J. M. (2019). The role of iron regulation in immunometabolism and immune-related disease. *Frontiers in Molecular Biosciences*, 6, 116.
- Cullis, J. (2013). Anaemia of chronic disease. *Clinical Medicine*, 13(2), 193–196.
- Cullis, J. O. (2011). Diagnosis and management of anaemia of chronic disease: Current status. *British Journal of Haematology*, 154(3), 289–300.
- D'alessandro, A., Dzieciatowska, M., Nemkov, T., & Hansen, K. C. (2017). Red blood cell proteomics update: Is there more to discover? *Blood transfusion = Trasfusione del sangue*, 15(2), 182–187.
- Darbari, D. S., Sheehan, V. A., & Ballas, S. K. (2020). The vaso-occlusive pain crisis in sickle cell disease: Definition, pathophysiology, and management. *European Journal of Haematology*, 105(3), 237–246.
- DeLoughery, T. G. (2017). Iron deficiency anemia. *Medical Clinics of North America*, 101(2), 319–332.
- De Domenico, I., McVey Ward, D., Nemeth, E., Ganz, T., Corradini, E., Ferrara, F., Musci, G., Pietrangelo, A., & Kaplan, J. (2006). Molecular and clinical correlates in iron overload associated with mutations in ferroportin. *Haematologica*, 91(8), 1092–1095.
- De Domenico, I., Ward, D. M., Langelier, C., Vaughn, M. B., Nemeth, E., Sundquist, W. I., Ganz, T., Musci, G., & Kaplan, J. (2007). The molecular mechanism of hepcidin-mediated ferroportin down-regulation. *Molecular Biology of the Cell*, 18(7), 2569–2578.
- Drakesmith, H., & Prentice, A. M. (2012). Hepcidin and the iron-infection axis. *Science*, 338(6108), 768–772.
- Eaton, W. A., & Bunn, H. F. (2017). Treating sickle cell disease by targeting HbS polymerization. *Blood*, 129(20), 2719–2726.
- Eaton, W. A., Henry, E. R., Hofrichter, J., Bettati, S., Viappiani, C., & Mozzarelli, A. (2007). Evolution of allosteric models for hemoglobin. *IUBMB Life*, 59(8-9), 586–599.
- Eaton, W. A., Henry, E. R., Hofrichter, J., & Mozzarelli, A. (2006). Is cooperative oxygen binding by hemoglobin really understood? *Rendiconti Lincei*, 17(1), 147–162.
- Eldeniz, C., Binkley, M. M., Fields, M., Williams, K., Ragan, D. K., Chen, Y., Lee, J. M., Ford, A. L., & An, H. (2021). Bulk volume susceptibility difference between deoxyhemoglobin and oxyhemoglobin for HbA and HbS: A comparative study. *Magnetic Resonance in Medicine*, 85(6), 3383–3393.
- Elsayed, M., Sharif, M., & Stack, A. (2016). Transferrin saturation: A body iron biomarker. *Advances in Clinical Chemistry*, 75, 71–97.
- Fabry, M. E., Mears, J. G., Patel, P., Schaefer-Rego, K., Carmichael, L. D., Martinez, G., & Nagel, R. L. (1984). *Dense cells in sickle cell anemia: The effects of gene interaction.*
- Feldman, B. V., Zinkl, J. G., Jain, N. C., & Schalm, O. W. (2000). In Bernard V. Feldman, Joseph G. Zinkl, & Nemi C. Jain (Eds.), *Schalm's veterinary hematology*. Lippincott Williams & Wilkins.
- Feng, P., Yang, Q., Luo, L., Sun, Y., Lv, W., Wan, S., Guan, Z., Xiao, Z., Liu, F., Li, Z., Dong, Z., & Yang, M. (2021). The kinase PDK1 regulates regulatory T cell survival via controlling redox homeostasis. *Theranostics*, 11(19), 9503–9518.
- Fillet, G., Beguin, Y., & Baldelli, L. (1989). *Model of reticuloendothelial iron metabolism in humans: Abnormal behavior in idiopathic hemochromatosis and in inflammation.*
- Fishman, S. M., Christian, P., & West, K. P. (2000). The role of vitamins in the prevention and control of anaemia. *Public Health Nutrition*, 3(2), 125–150.
- Flo, T. H., Smith, K. D., Sato, S., Rodriguez, D. J., Holmes, M. A., Strong, R. K., Akira, S., & Aderem, A. (2004). Lipocalin 2 mediates an

- innate immune response to bacterial infection by sequestering iron. *Nature*, 432(7019), 917–921.
- Fraenkel, P. G. (2015). Understanding anemia of chronic disease. *Hematology*, 2015(1), 14–18.
- Furlani, E. P. (2007). Magnetophoretic separation of blood cells at the microscale. *Journal of Physics D: Applied Physics*, 40(5), 1313–1319.
- Galanello, R., & Origa, R. (2010). Beta-thalassemia. *Orphanet Journal of Rare Diseases*, 5(1), 11.
- Ganz, T., & Nemeth, E. (2015). Iron homeostasis in host defence and inflammation. *Nature Reviews Immunology*, 15(8), 500–510.
- Ganz, T., & Nemeth, E. (2006). Iron imports. IV. Hepcidin and regulation of body iron metabolism. *American Journal of Physiology-Gastrointestinal and Liver Physiology*, 290(2), G199–G203.
- Gao, J., & Monaghan, S. A. (2018). Red blood cell/hemoglobin disorders, in *Hematopathology* (pp. 3–56. e2). Elsevier.
- Garzon, S., Cacciato, P. M., Certelli, C., Salvaggio, C., Magliarditi, M., & Rizzo, G. (2020). Iron deficiency anemia in pregnancy: Novel approaches for an old problem. *Oman Medical Journal*, 35(5), e166.
- Gattermann, N. (2009). The treatment of secondary hemochromatosis. *Deutsches Ärzteblatt international*, 106(30), 499.
- Ge, S., Nemiroski, A., Mirica, K. A., Mace, C. R., Hennek, J. W., Kumar, A. A., & Whitesides, G. M. (2020). Magnetic levitation in chemistry, materials science, and biochemistry. *Angewandte Chemie International Edition*, 59(41), 17810–17855.
- Ge, S., & Whitesides, G. M. (2018). “Axial” magnetic levitation using ring magnets enables simple density-based analysis, separation, and manipulation. *Analytical Chemistry*, 90(20), 12239–12245.
- Ghlichloo, I., & Gerriets, V. (2019). *Nonsteroidal anti-inflammatory drugs (NSAIDs)*.
- Gijjs, M. A. M., Lacharme, F., & Lehmann, U. (2010). Microfluidic applications of magnetic particles for biological analysis and catalysis. *Chemical Reviews*, 110(3), 1518–1563.
- Girelli, D., Busti, F., Brissot, P., Cabantchik, I., Muckenthaler, M. U., & Porto, G. (2022). Hemochromatosis classification: Update and recommendations by the BIOIRON society. *Blood*, 139(20), 3018–3029.
- Golubovskaya, V., & Wu, L. (2016). Different subsets of T cells, memory, effector functions, and CAR-T immunotherapy. *Cancers*, 8(3), 36.
- Gómez-Pastora, J., Bringas Elizalde, E., & Ortiz Uribe, I. (2016). *Design of novel adsorption processes for the removal of arsenic from polluted groundwater employing functionalized magnetic nanoparticles*.
- Gómez-Pastora, J., Moore, L.R., Chalmers, J.J., & Zborowski, M. (2022). Magnetic separations, *Particle Separation Techniques* (pp. 221–266). Elsevier.
- Gómez-Pastora, J., Kim, J., Multanen, V., Weigand, M., Walters, N. A., Reátegui, E., Palmer, A. F., Yazer, M. H., Zborowski, M., & Chalmers, J. J. (2021). Intrinsically magnetic susceptibility in human blood and its potential impact on cell separation: Non-classical and intermediate monocytes have the strongest magnetic behavior in fresh human blood. *Experimental Hematology*, 99, 21–31.
- Gómez-Pastora, J., Bringas, E., Lázaro-Díez, M., Ramos-Vivas, J., & Ortiz, I. (2018). The reverse of controlled release: Controlled sequestration of species and biotoxins into nanoparticles (NPs). In P. Stroeve & M. Mahmoudi (Eds.), *Drug Delivery Systems* (pp. 207–244).
- Gómez-Pastora, J., Weigand, M., Kim, J., Palmer, A. F., Yazer, M., Desai, P. C., Zborowski, M., & Chalmers, J. J. (2022). Potential of cell tracking velocimetry as an economical and portable hematology analyzer. *Scientific Reports*, 12(1), 1692.
- Gómez-Pastora, J., Wu, X., & Chalmers, J. J. (2022). *Magnetic Separation of Micro-and Nanoparticles for Water Treatment Processes, in Solid-Liquid Separation Technologies* (pp. 211–232). CRC Press.
- Gómez-Pastora, J., Wu, X., Sundar, N., Alawi, J., Nabar, G., Winter, J. O., Zborowski, M., & Chalmers, J. J. (2020). Self-assembly and sedimentation of 5 nm SPIONs using horizontal, high magnetic fields and gradients. *Separation and Purification Technology*, 248, 117012.
- Gómez-Pastora, J., Xue, X., Karampelas, I. H., Bringas, E., Furlani, E. P., & Ortiz, I. (2017). Analysis of separators for magnetic beads recovery: From large systems to multifunctional microdevices. *Separation and Purification Technology*, 172, 16–31.
- González-Fernández, C., Gómez Pastora, J., Basauri, A., Fallanza, M., Bringas, E., Chalmers, J. J., & Ortiz, I. (2020). Continuous-flow separation of magnetic particles from biofluids: How does the microdevice geometry determine the separation performance? *Sensors*, 20(11), 3030.
- González-Fernández, C., Gómez-Pastora, J., Bringas, E., Zborowski, M., Chalmers, J. J., & Ortiz, I. (2021). Recovery of magnetic catalysts: Advanced design for process intensification. *Industrial & Engineering Chemistry Research*, 60(46), 16780–16790.
- Gordon-Smith, T. (2013). Structure and function of red and white blood cells. *Medicine*, 41(4), 193–199.
- Gupta, D. C. P. (2014). Role of iron (Fe) in body. *IOSR Journal of Applied Chemistry*, 7(11), 38–46.
- Guralnik, J. M., Eisenstaedt, R. S., Ferrucci, L., Klein, H. G., & Woodman, R. C. (2004). Prevalence of anemia in persons 65 years and older in the United States: Evidence for a high rate of unexplained anemia. *Blood*, 104(8), 2263–2268.
- Ha, C. E., & Bhagavan, N. V. (2015). Hemoglobin, in *Essentials of Medical Biochemistry: With Clinical Cases* (pp. 489–509). Elsevier Science.
- Han, K.-H., & Frazier, A. B. (2006). Paramagnetic capture mode magnetophoretic microseparator for high efficiency blood cell separations. *Lab on a Chip*, 6(2), 265–273.
- Han, X., Feng, Y., Cao, Q., & Li, L. (2015). Three-dimensional analysis and enhancement of continuous magnetic separation of particles in microfluidics. *Microfluidics and Nanofluidics*, 18(5), 1209–1220.
- Harewood, J., & Azevedo, A. M., Alpha Thalassemia. 2017.
- Harteveld, C. L., & Higgs, D. R. (2010).  $\alpha$ -thalassaemia. *Orphanet Journal of Rare Diseases*, 5(1), 13.
- Hasan, M. N., Fraiwan, A., Little, J.A., & Gurkan, U.A. (2017). A low-cost, mass-producible point-of-care platform for diagnosing hemoglobin disorders, *IEEE Healthcare Innovations and Point of Care Technologies (HI-POCT)*. IEEE.
- Haschka, D., Petzer, V., Kocher, F., Tschurtschenthaler, C., Schaefer, B., Seifert, M., Sopper, S., Sonnweber, T., Feistritzer, C., Arvedson, T. L., Zoller, H., Stauder, R., Theurl, I., Weiss, G., & Tymoszyk, P. (2019). Classical and intermediate monocytes scavenge non-transferrin-bound iron and damaged erythrocytes. *JCI Insight*, 4(8), e98867.
- Hassan, T. H., Badr, M. A., Karam, N. A., Zkaria, M., El Saadany, H. F., Abdel Rahman, D. M., Shahbah, D. A., Al Morshedy, S. M., Fathy, M., Esh, A. M. H., & Selim, A. M. (2016). Impact of iron deficiency anemia on the function of the immune system in children. *Medicine*, 95(47), e5395.
- Heikali, D., & Di, D. (2010). Carlo, A niche for microfluidics in portable hematology analyzers. *JALA: Journal of the Association for Laboratory Automation*, 15(4), 319–328.
- Hejazian, M., Li, W., & Nguyen, N.-T. (2015). Lab on a chip for continuous-flow magnetic cell separation. *Lab on a Chip*, 15(4), 959–970.
- Helmi, N., Bashir, M., Shireen, A., & Mirza Ahmed, I. (2017). Thalassemia review: Features, dental considerations and management. *Electronic Physician*, 9(3), 4003–4008.
- Hematology, A. S. o (2016). *Rare patients with sickle cell disease live nearly twice as long as average*. Retrieved July 13, 2022, from <https://www.hematology.org/newsroom/press-releases/2016/rare-patients-with-sickle-cell-disease-live-nearly-twice-as-long-as-average>
- Hematology, A. S. O. *Sickle Cell Trait*. <https://www.hematology.org/education/patients/anemia/sickle-cell-trait>
- Hentze, M. W., Muckenthaler, M. U., & Andrews, N. C. (2004). Balancing acts. *Cell*, 117(3), 285–297.



- Hercberg, S., & Rouaud, C. (1981). Nutritional anaemia. *Children in the Tropics*, 133, 1–36.
- Hider, R. C. (2002). Nature of nontransferrin-bound iron. *European Journal of Clinical Investigation*, 32, 50–54.
- Hider, R. C., & Kong, X. (2013). Iron: Effect of overload and deficiency. *Interrelations Between Essential Metal Ions and Human Diseases*, 13, 229–294.
- Howard, J. (2016). Sick cell disease: When and how to transfuse. *Hematology*, 2016(1), 625–631.
- Huang, T.-L., Zhang, T. Y., Song, C. Y., Lin, Y. B., Sang, B. H., Lei, Q. L., Lv, Y., Yang, C. H., Li, N., Tian, X., Yang, Y. H., & Zhang, X. W. (2020). Gene mutation spectrum of thalassemia among children in Yunnan province. *Frontiers in Pediatrics*, 8, 159.
- Hudig, D., Hunter, K. W., Diamond, W. J., & Redelman, D. (2014). Properties of human blood monocytes. I. CD91 expression and log orthogonal light scatter provide a robust method to identify monocytes that is more accurate than CD14 expression. *Cytometry Part B: Clinical Cytometry*, 86(2), 111–120.
- Hvidberg, V., Maniecki, M. B., Jacobsen, C., Højrup, P., Møller, H. J., & Moestrup, S. K. (2005). Identification of the receptor scavenging hemopexin-heme complexes. *Blood*, 106(7), 2572–2579.
- Ilyas, S., Simonson, A. E., & Asghar, W. (2020). Emerging point-of-care technologies for sickle cell disease diagnostics. *Clinica Chimica Acta*, 501, 85–91.
- Institute, N. C. (2022). *White blood cell*. Retrieved March 26, 2022, from <https://www.cancer.gov/publications/dictionaries/cancer-terms/def/white-blood-cell>
- Inusa, B., Hsu, L., Kohli, N., Patel, A., Ominu-Evbota, K., Anie, K., & Atoyebi, W. (2019). Sick cell disease—genetics, pathophysiology, clinical presentation and treatment. *International Journal of Neonatal Screening*, 5(2), 20.
- Jacobs, J. W., & Booth, G. S. (2022). Blood shortages and changes to massive transfusion protocols: Survey of hospital practices during the COVID-19 pandemic. *Transfusion and Apheresis Science*, 61(1), 103297.
- Jang, T., Poplowska, M., Cimpeanu, E., Mo, G., Dutta, D., & Lim, S. H. (2021). Vaso-occlusive crisis in sickle cell disease: A vicious cycle of secondary events. *Journal of Translational Medicine*, 19(1), 397.
- Jensen, F. B., Fago, A., & Weber, R. E. (1998). Hemoglobin structure and function. *Fish physiology*, 17, 1–40.
- Jiang, Y., Li, C., Wu, Q., An, P., Huang, L., Wang, J., Chen, C., Chen, X., Zhang, F., Ma, L., Liu, S., He, H., Xie, S., Sun, Y., Liu, H., Zhan, Y., Tao, Y., Liu, Z., Sun, X., ... Zhang, X. (2019). Iron-dependent histone 3 lysine 9 demethylation controls B cell proliferation and humoral immune responses. *Nature Communications*, 10(1), 2935.
- Johnson-Wimbley, T. D., & Graham, D. Y. (2011). Diagnosis and management of iron deficiency anemia in the 21st century. *Therapeutic Advances in Gastroenterology*, 4(3), 177–184.
- Karampelas, I. H., & Gómez-Pastora, J. (2022). Novel approaches concerning the numerical modeling of particle and cell separation in microchannels: A review. *Processes*, 10(6), 1226.
- Kim, J., Gómez-Pastora, J., Gilbert, C. J., Weigand, M., Walters, N. A., Reátegui, E., Palmer, A. F., Yazer, M., Zborowski, M., & Chalmers, J. J. (2019). Quantification of the mean and distribution of hemoglobin content in normal human Ablood using cell tracking velocimetry. *Analytical Chemistry*, 92(2), 1956–1962.
- Kim, J., Gómez-Pastora, J., Weigand, M., Potgieter, M., A. Walters, N., Reátegui, E., F. Palmer, A., Yazer, M., Zborowski, M., & Chalmers, J. J. (2019). A subpopulation of monocytes in normal human blood has significant magnetic susceptibility: Quantification and potential implications. *Cytometry, Part A*, 95(5), 478–487.
- Klinken, S. P. (2002). Red blood cells. *The International Journal of Biochemistry & Cell Biology*, 34(12), 1513–1518.
- Knobloch, J., Casjens, S., Lehnert, M., Yanik, S. D., Körber, S., Lotz, A., Rupp, J., Raulf, M., Zschesche, W., Weiss, T., Kronsbein, J., Koch, A., Brüning, T., & Pesch, B. (2020). Exposure to welding fumes suppresses the activity of T-helper cells. *Environmental Research*, 189, 109913.
- Knowlton, S. M., Sencan, I., Aydar, Y., Khoory, J., Heeney, M. M., Ghiran, I. C., & Tasoglu, S. (2015). Sick cell detection using a smartphone. *Scientific Reports*, 5(1), 15022.
- Koch, A., Yang, Q., & Olney, R. (2000). Sick hemoglobin allele and sickle cell disease: A HuGE review. *American Journal of Epidemiology*, 151(9), 839–845.
- Kumar, A. A., Chunda-Liyoka, C., Hennek, J. W., Mantina, H., Lee, S. Y. R., Patton, M. R., Sambo, P., Sinyangwe, S., Kankasa, C., Chintu, C., Brugnara, C., Stossel, T. P., & Whitesides, G. M. (2014). Evaluation of a density-based rapid diagnostic test for sickle cell disease in a clinical setting in Zambia. *PLoS One*, 9(12), e114540.
- Kumar, A. A., Patton, M. R., Hennek, J. W., Lee, S. Y. R., D'Alesio-Spina, G., Yang, X., Kanter, J., Shevkopyas, S. S., Brugnara, C., & Whitesides, G. M. (2014). Density-based separation in multiphase systems provides a simple method to identify sickle cell disease. *Proceedings of the National Academy of Sciences*, 111(41), 14864–14869.
- LaRosa, D., & Orange, J. (2008). 1. Lymphocytes. *Journal of Allergy and Clinical Immunology*, 121(2), S364–S369.
- Lee, P., Peng, H., Gelbart, T., Wang, L., & Beutler, E. (2005). Regulation of hepcidin transcription by interleukin-1 and interleukin-6. *Proceedings of the National Academy of Sciences*, 102(6), 1906–1910.
- Lenhof, A., & Laurell, T. (2010). Continuous separation of cells and particles in microfluidic systems. *Chemical Society Reviews*, 39(3), 1203–1217.
- Li, C.-K. (2017). New trend in the epidemiology of thalassaemia. *Best Practice & Research Clinical Obstetrics & Gynaecology*, 39, 16–26.
- Lima Santos, P. C. J., Dinardo, C. L., Cançado, R. D., Schettter, I. T., Krieger, J. E., & Pereira, A. C. (2012). Non-HFE hemochromatosis. *Revista Brasileira de Hematologia e Hemoterapia*, 34, 311–316.
- Littwitz-Salomon, E., Moreira, D., Frost, J. N., Choi, C., Liou, K. T., Ahern, D. K., O'Shaughnessy, S., Wagner, B., Biron, C. A., Drakesmith, H., Dittmer, U., & Finlay, D. K. (2021). Metabolic requirements of NK cells during the acute response against retroviral infection. *Nature Communications*, 12(1), 5376.
- Lonergan, G. J., Cline, D. B., & Abbondanzo, S. L. (2001). Sick cell anemia. *Radiographics*, 21(4), 971–994.
- Lopez, A., Cacoub, P., Macdougall, I. C., & Peyrin-Biroulet, L. (2016). Iron deficiency anaemia. *The Lancet*, 387(10021), 907–916.
- Lu, J., Basu, A., Melenhorst, J. J., Young, N. S., & Brown, K. E. (2004). Analysis of T-cell repertoire in hepatitis-associated aplastic anemia. *Blood*, 103(12), 4588–4593.
- Ludwiczek, S., Aigner, E., Theurl, I., & Weiss, G. (2003). Cytokine-mediated regulation of iron transport in human monocytic cells. *Blood*, 101(10), 4148–4154.
- Macedo, M. F., De Sousa, M., Ned, R. M., Mascarenhas, C., Andrews, N. C., & Correia-Neves, M. (2004). Transferrin is required for early T-cell differentiation. *Immunology*, 112(4), 543–549.
- Mahabadi, N., Bhusal, A., & Banks, S. W. (2022). *Riboflavin Deficiency*, in *StatPearls [Internet]*. StatPearls Publishing.
- Mairbäur, H. (2013). Red blood cells in sports: Effects of exercise and training on oxygen supply by red blood cells. *Frontiers in Physiology*, 4, 332.
- Marengo-Rowe, A. J. (2007). The thalassemias and related disorders. *Baylor university medical center proceedings*. Taylor & Francis.
- Mburu, J., & Odame, I. (2019). Sick cell disease: Reducing the global disease burden. *International journal of laboratory hematology*, 41, 82–88.
- McCloskey, K. E., Chalmers, J. J., & Zborowski, M. (2003). Magnetic cell separation: Characterization of magnetophoretic mobility. *Analytical Chemistry*, 75(24), 6868–6874.
- McLaren, G. D. (1989). Reticuloendothelial iron stores and hereditary hemochromatosis: A paradox. *The Journal of Laboratory and Clinical Medicine*, 113(2), 137–138.



- Means Jr. RT. (2003). Recent developments in the anemia of chronic disease. *Current hematology reports*, 2(2), 116–121.
- Mehta, S. R., Afenyi-Annan, A., Byrns, P. J., & Lottenberg, R. (2006). Opportunities to improve outcomes in sickle cell disease. *American Family Physician*, 74(2), 303–310.
- Meier, P. J., Giger, U., Brändli, O., & Fehr, J. (1981). [Acquired, vitamin B6-responsive, primary sideroblastic anemia, an enzyme deficiency in heme synthesis]. *Schweizerische Medizinische Wochenschrift*, 111(41), 1533–1535.
- Miller, J. L. (2013). Iron deficiency anemia: A common and curable disease. *Cold Spring Harbor Perspectives in Medicine*, 3(7), a011866.
- Milman, N. T., Schioedt, F. V., Junker, A. E., & Magnussen, K. (2019). Diagnosis and treatment of genetic HFE-hemochromatosis: The Danish aspect. *Gastroenterology Research*, 12(5), 221–232.
- Mirica, K. A., Shevkopyas, S. S., Phillips, S. T., Gupta, M., & Whitesides, G. M. (2009). Measuring densities of solids and liquids using magnetic levitation: Fundamentals. *Journal of the American Chemical Society*, 131(29), 10049–10058.
- Monod, J., Wyman, J., & Changeux, J. P. (1965). On the nature of allosteric transitions: A plausible model. *Journal of Molecular Biology*, 12, 88–118.
- Moura, E., Noordermeer, M. A., Verhoeven, N., Verheul, A. F. M., Marx, J. J. M., Moura, E., Noordermeer, M. A., Verhoeven, N., Verheul, A. F. M., & Marx, J. J. M. (1998). Iron release from human monocytes after erythrophagocytosis in vitro: An investigation in normal subjects and hereditary hemochromatosis patients. *Blood*, 92(7), 2511–2519.
- Munaz, A., Shiddiky, M. J. A., & Nguyen, N.-T. (2018). Magnetophoretic separation of diamagnetic particles through parallel ferrofluid streams. *Sensors and Actuators B: Chemical*, 275, 459–469.
- Muncie Jr, H. L., & Campbell, J. (2009). Alpha and beta thalassemia. *American Family Physician*, 80(4), 339–344.
- Murphree, C. R., Nguyen, N. N., Raghunathan, V., Olson, S. R., DeLoughery, T., & Shatzel, J. J. (2020). Diagnosis and management of hereditary haemochromatosis. *Vox Sanguinis*, 115(4), 255–262.
- Nagababu, E., Gulyani, S., Earley, C. J., Cutler, R. G., Mattson, M. P., & Rifkind, J. M. (2008). Iron-deficiency anaemia enhances red blood cell oxidative stress. *Free Radical Research*, 42(9), 824–829.
- Naigamwalla, D. Z., Webb, J. A., & Giger, U. (2012). Iron deficiency anemia. *The Canadian Veterinary Journal*, 53(3), 250.
- Nakashige, T. G., Zhang, B., Krebs, C., & Nolan, E. M. (2015). Human calprotectin is an iron-sequestering host-defense protein. *Nature Chemical Biology*, 11(10), 765–771.
- Nemeth, E., & Ganz, T. (2014). Anemia of inflammation. *Hematology/Oncology Clinics of North America*, 28(4), 671–681.
- Nemeth, E., Tuttle, M. S., Powelson, J., Vaughn, M. B., Donovan, A., Ward, D. M., Ganz, T., & Kaplan, J. (2004). Hepcidin regulates cellular iron efflux by binding to ferroportin and inducing its internalization. *Science*, 306(5704), 2090–2093.
- Neto, L. M. M., Zufelato, N., de Sousa-Júnior, A. A., Trentini, M. M., da Costa, A. C., Bakuzis, A. F., Kipnis, A., & Junqueira-Kipnis, A. P. (2018). Specific T cell induction using iron oxide based nanoparticles as subunit vaccine adjuvant. *Human Vaccines & Immunotherapeutics*, 14(11), 2786–2801.
- Ngolet, L. O., Moyen Engoba, M., Kocko, I., Elira Dokekias, A., Mombouli, J. V., & Moyen, G. M. (2016). Sickle-cell disease healthcare cost in Africa: Experience of the Congo. *Anemia*, 2016, 1–5.
- Ni, S., Yuan, Y., Kuang, Y., & Li, X. (2022). Iron metabolism and immune regulation. *Frontiers in Immunology*, 13, 816282.
- Niederau, C., Fischer, R., Purschel, A., Stremmel, W., Haussinger, D., & Strohmeyer, G. (1996). Long-term survival in patients with hereditary hemochromatosis. *Gastroenterology*, 110(4), 1107–1119.
- Oppenheimer, S. J. (2001). Iron and its relation to immunity and infectious disease. *The Journal of Nutrition*, 131(2), 616S–635S.
- Pamme, N., & Manz, A. (2004). On-chip free-flow magnetophoresis: Continuous flow separation of magnetic particles and agglomerates. *Analytical Chemistry*, 76(24), 7250–7256.
- Pasricha, S.-R., Tye-Din, J., Muckenthaler, M. U., & Swinkels, D. W. (2021). Iron deficiency. *The Lancet*, 397(10270), 233–248.
- Pauling, L., & Coryell, C. D. (1936a). The magnetic properties and structure of hemoglobin, oxyhemoglobin and carbonmonoxyhemoglobin. *Proceedings of the National Academy of Sciences*, 22(4), 210–216.
- Pauling, L., & Coryell, C. D. (1936b). The magnetic properties and structure of the hemochromogens and related substances. *Proceedings of the National Academy of Sciences*, 22(3), 159–163.
- Perutz, M. F. (1970). Stereochemistry of cooperative effects in haemoglobin: Haem-haem interaction and the problem of allostery. *Nature*, 228(5273), 726–734.
- Perutz, M. F. (1979). Regulation of oxygen affinity of hemoglobin: Influence of structure of the globin on the heme iron. *Annual Review of Biochemistry*, 48(1), 327–386.
- Phatak, P., Brissot, P., Wurster, M., Adams, P. C., Bonkovsky, H. L., Gross, J., Malfertheiner, P., McLaren, G. D., Niederau, C., Piperno, A., Powell, L. W., Russo, M. W., Stoelzel, U., Stremmel, W., Griffel, L., Lynch, N., Zhang, Y., & Pietrangelo, A. (2010). A phase 1/2, dose-escalation trial of deferasirox for the treatment of iron overload in HFE-related hereditary hemochromatosis. *Hepatology*, 52(5), 1671–1779.
- Pietrangelo, A. (2004). The ferroportin disease. *Blood Cells, Molecules, and Diseases*, 32(1), 131–138.
- Platt, O. S., Orkin, S. H., Dover, G., Beardsley, G. P., Miller, B., & Nathan, D. G. (1984). Hydroxyurea enhances fetal hemoglobin production in sickle cell anemia. *Journal of Clinical Investigation*, 74(2), 652–656.
- Porter, J. L., & Rawla, P. (2021). *Hemochromatosis*, in *StatPearls [Internet]*. StatPearls Publishing.
- Powell, L. W., Dixon, J. L., Ramm, G. A., Purdie, D. M., Lincoln, D. J., Anderson, G. J., Subramaniam, V. N., Hewett, D. G., Searle, J. W., Fletcher, L. M., Crawford, D. H., Rodgers, H., Allen, K. J., Cavanaugh, J. A., & Bassett, M. L. (2006). Screening for hemochromatosis in asymptomatic subjects with or without a family history. *Archives of Internal Medicine*, 166(3), 294–301.
- Quirolo, K., Bertolone, S., Hassell, K., Howard, T., King, K. E., Rhodes, D. K., & Bill, J. (2015). The evaluation of a new apheresis device for automated red blood cell exchange procedures in patients with sickle cell disease. *Transfusion*, 55(4), 775–781.
- Renassia, C., Louis, S., Cuvelier, S., Boussetta, N., Deschemin, J. C., Borderie, D., Bailly, K., Poupon, J., Dang, P. M. C., El-Benna, J., Manceau, S., Lefrère, F., Vaulont, S., & Peyssonnaud, C. (2020). Neutrophils from hereditary hemochromatosis patients are protected from iron excess and are primed. *Blood Advances*, 4(16), 3853–3863.
- Robert, D., Pamme, N., Conjeaud, H., Gazeau, F., Iles, A., & Wilhelm, C. (2011). Cell sorting by endocytotic capacity in a microfluidic magnetophoresis device. *Lab on a Chip*, 11(11), 1902–1910.
- Roetto, A., Papanikolaou, G., Politou, M., Alberti, F., Girelli, D., Christakis, J., Loukopoulou, D., & Camaschella, C. (2003). Mutant antimicrobial peptide hepcidin is associated with severe juvenile hemochromatosis. *Nature Genetics*, 33(1), 21–22.
- Roetto, A., Totaro, A., Piperno, A., Piga, A., Longo, F., Garozzo, G., Cali, A., De Gobbi, M., Gasparini, P., & Camaschella, C. (2001). New mutations inactivating transferrin receptor 2 in hemochromatosis type 3. *Blood*, 97(9), 2555–2560.
- Ross, S. H., & Cantrell, D. A. (2018). Signaling and function of interleukin-2 in T lymphocytes. *Annual Review of Immunology*, 36, 411–433.
- Ross, W. B., & Yap, P. L. (1990). Blood transfusion and organ transplantation. *Blood Reviews*, 4(4), 252–258.
- Rubio-Navarro, A., Amaro Villalobos, J. M., Lindholt, J. S., Buendía, I., Egido, J., Blanco-Colio, L. M., Samaniego, R., Meilhac, O., Michel, J. B., Martín-

- Ventura, J. L., & Moreno, J. A. (2015). Hemoglobin induces monocyte recruitment and CD163-macrophage polarization in abdominal aortic aneurysm. *International Journal of Cardiology*, 201, 66–78.
- Rund, D., & Rachmilewitz, E. (2001). Pathophysiology of  $\alpha$ - and  $\beta$ -thalassemia: Therapeutic implications, *Seminars in hematology*. Elsevier.
- Sahlol, A. T., Kollmannsberger, P., & Ewees, A. A. (2020). Efficient classification of white blood cell leukemia with improved swarm optimization of deep features. *Scientific Reports*, 10(1), 2536.
- Sarma, P. R. (1990). *Red cell indices. Clinical methods: The history, physical, and laboratory examinations*. 3rd edition.
- Sauls, R. S., McCausland, C., & Taylor, B. N. (2018). *Histology, T-Cell Lymphocyte*.
- Schaefer, R. M., & Schaefer, L. (1999). Hypochromic red blood cells and reticulocytes. *Kidney International*, 55, S44–S48.
- Scott, M. A., & Stockham, S. L. (2013). *Fundamentals of veterinary clinical pathology*. John Wiley & Sons.
- Scrima, R., Fugetto, S., Capitanio, N., & Gatti, D. L. (2019). Hemoglobin non-equilibrium oxygen dissociation curve. *arXiv preprint arXiv: 2001.00091*.
- Scrimshaw, N., & SanGiovanni, J. (1997). Synergism of nutrition, infection, and immunity: An overview. *The American Journal of Clinical Nutrition*, 66(2), 464S–477S.
- Semba, R., & Bloem, M. (2002). The anemia of vitamin A deficiency: Epidemiology and pathogenesis. *European Journal of Clinical Nutrition*, 56(4), 271–281.
- Shafique, F., Ali, S., Almansouri, T., Van Eeden, F., Shafi, N., Khalid, M., Khawaja, S., & Andleeb, S. (2021). Thalassemia, a human blood disorder. *Brazilian Journal of Biology*, 83, e246062.
- Shah, N., Bhor, M., Xie, L., Paulose, J., & Yuce, H. (2019). Sickle cell disease complications: Prevalence and resource utilization. *PLoS One*, 14(7), e0214355.
- Shiriny, A., & Bayareh, M. (2020). On magnetophoretic separation of blood cells using Halbach array of magnets. *Meccanica*, 55(10), 1903–1916.
- Siddique, A., & Kowdley, K. V. (2012). Review article: The iron overload syndromes. *Alimentary Pharmacology & Therapeutics*, 35(8), 876–893.
- Siswandari, W., Rujito, L., Indriani, V., & Djatmiko, W. (2019). *Mentzer Index Diagnostic Value in Predicting Thalassemia Diagnosis IOP Conference Series: Earth and Environmental Science*. IOP Publishing.
- Smith, J. A., & Kinney, T. R. (1997). *Sickle cell disease: Screening, diagnosis, management, and counseling in newborns and infants*. DIANE Publishing.
- Soares, M. P., & Hamza, I. (2016). Macrophages and iron metabolism. *Immunity*, 44(3), 492–504.
- Song, Y., Huang, Y. Y., Liu, X., Zhang, X., Ferrari, M., & Qin, L. (2014). Point-of-care technologies for molecular diagnostics using a drop of blood. *Trends in Biotechnology*, 32(3), 132–139.
- Songdej, D., & Fucharoen, S. (2022). Alpha-Thalassemia: Diversity of clinical phenotypes and update on the treatment. *Thalassemia Reports*, 12(4), 157–172.
- Sukhbaatar, N., & Weichhart, T. (2018). Iron regulation: Macrophages in control. *Pharmaceuticals*, 11(4), 137.
- Sullivan, E. (2006). Hematology analyzer: From workhorse to thoroughbred. *Laboratory Medicine*, 37(5), 273–278.
- Tarn, M. D., & Pamme, N. (2017). On-chip magnetic particle-based immunoassays using multilaminar flow for clinical diagnostics, *Microchip Diagnostics* (pp. 69–83). Springer.
- Telen, M. J. (2001). Principles and problems of transfusion in sickle cell disease, *Seminars in Hematology*. Elsevier.
- Thomas, C., & Lumb, A. B. (2012). Physiology of haemoglobin. continuing education in anaesthesia. *Critical Care and Pain*, 12(5), 251–256.
- Tisdale, J. F., Thein, S. L., & Eaton, W. A. (2020). Treating sickle cell anemia. *Science*, 367(6483), 1198–1199.
- Vichinsky, E. (2012). Advances in the treatment of alpha-thalassemia. *Blood Reviews*, 26, S31–S34.
- Vij, R., & Machado, R. F. (2010). Pulmonary complications of hemoglobinopathies. *Chest*, 138(4), 973–983.
- Wallace, D. F. (2007). Non-HFE haemochromatosis. *World Journal of Gastroenterology*, 13(35), 4690.
- Walsh, T. S., & Saleh, E.-E.-D. (2006). Anaemia during critical illness. *British Journal of Anaesthesia*, 97(3), 278–291.
- Wang, S., Cheng, M., Peng, P., Lou, Y., Zhang, A., & Liu, P. (2021). Iron released after cryo-thermal therapy induced M1 macrophage polarization, promoting the differentiation of CD4+ T cells into CTLs. *International Journal of Molecular Sciences*, 22(13), 7010.
- Ware, R. E., de Montalembert, M., Tshililo, L., & Abboud, M. R. (2017). Sickle cell disease. *The Lancet*, 390(10091), 311–323.
- Watarai, H., & Namba, M. (2002). Capillary magnetophoresis of human blood cells and their magnetophoretic trapping in a flow system. *Journal of Chromatography A*, 961(1), 3–8.
- Webster, J. G., Zborowski, M., & Chalmers, J. J. (2015). Magnetophoresis: Fundamentals and applications. In J. G. Webster (Ed.), *Wiley encyclopedia of electrical and electronics engineering*. <https://doi.org/10.1002/047134608X.W8236>
- Weigand, M., Gomez-Pastora, J., Palmer, A., Zborowski, M., Desai, P., & Chalmers, J. (2022). Continuous-Flow magnetic fractionation of red blood cells based on hemoglobin content and oxygen Saturation—Clinical blood supply implications and sickle cell anemia treatment. *Processes*, 10(5), 927.
- Weigand, M., Gomez-Pastora, J., Strayer, J., Wu, X., Choe, H., Lu, S., Plencner, E., Landes, K., Palmer, A., Zborowski, M., Desai, P., & Chalmers, J. (2022). The unique magnetic signature of sickle red blood cells: A comparison between the red blood cells of transfused and non-transfused sickle cell disease patients and healthy donors. *IEEE Transactions on Biomedical Engineering*, 69, 3582–3590.
- Weigand, M. R. H., Gómez-Pastora, J., Kim, J., Kurek, M. T., Hickey, R. J., Irwin, D. C., Buehler, P. W., Zborowski, M., Palmer, A. F., & Chalmers, J. J. (2021). Magnetophoretic and spectral characterization of oxyhemoglobin and deoxyhemoglobin: Chemical versus enzymatic processes. *PLoS One*, 16(9), e0257061.
- Weiss, G. (2002). Iron and immunity: A double-edged sword. *European Journal of Clinical Investigation*, 32, 70–78.
- Weiss, G. (2009). Iron metabolism in the anemia of chronic disease. *Biochimica et Biophysica Acta (BBA)-General Subjects*, 1790(7), 682–693.
- Weiss, G., & Goodnough, L. T. (2005). Anemia of chronic disease. *New England Journal of Medicine*, 352(10), 1011–1023.
- Whitesides, G. M. (2006). The origins and the future of microfluidics. *Nature*, 442(7101), 368–373.
- Wiciński, M., Licznar, G., Cadelski, K., Kołnierzak, T., Nowaczewska, M., & Malinowski, B. (2020). Anemia of chronic diseases: Wider diagnostics—better treatment? *Nutrients*, 12(6), 1784.
- Witte, D. L., Crosby, W. H., Edwards, C. Q., Fairbanks, V. F., & Mitros, F. A. (1996). Hereditary hemochromatosis. *Clinica Chimica Acta*, 245(2), 139–200.
- Wongrakpanich, S., Wongrakpanich, A., Melhado, K., & Rangaswami, J. (2018). A comprehensive review of non-steroidal anti-inflammatory drug use in the elderly. *Aging and Disease*, 9(1), 143.
- Wu, X., Gómez-Pastora, J., Zborowski, M., & Chalmers, J. (2022). SPIONs self-assembly and magnetic sedimentation in quadrupole magnets: Gaining insight into the separation mechanisms. *Separation and Purification Technology*, 280, 119786.
- Xu, J. Z., & Thein, S. L. (2019). The carrier state for sickle cell disease is not completely harmless. *Haematologica*, 104(6), 1106–1111.
- Xue, W., Moore, L. R., Nakano, N., Chalmers, J. J., & Zborowski, M. (2019). Single cell magnetometry by magnetophoresis vs. bulk cell suspension magnetometry by SQUID-MPMS—A comparison. *Journal of Magnetism and Magnetic Materials*, 474, 152–160.

- Yang, J., Zhang, L., Yu, C., Yang, X. F., & Wang, H. (2014). Monocyte and macrophage differentiation: Circulation inflammatory monocyte as biomarker for inflammatory diseases. *Biomarker Research*, 2(1), 1–9.
- Yiannikourides, A., & Latunde-Dada, G. (2019). A short review of iron metabolism and pathophysiology of iron disorders. *Medicines*, 6(3), 85.
- Yun, S., & Vincelette, N. D. (2015). Update on iron metabolism and molecular perspective of common genetic and acquired disorder, hemochromatosis. *Critical Reviews in Oncology/Hematology*, 95(1), 12–25.
- Yusuf, H. R., Lloyd-Puryear, M. A., Grant, A. M., Parker, C. S., Creary, M. S., & Atrash, H. K. (2011). Sickle cell disease. *American Journal of Preventive Medicine*, 41(6), S376–S383.
- Zborowski, M., Moore, L. R., Williams, P. S., & Chalmers, J. J. (2002). Separations based on magnetophoretic mobility. *Separation Science and Technology*, 37(16), 3611–3633.
- Zborowski, M., Ostera, G. R., Moore, L. R., Milliron, S., Chalmers, J. J., & Schechter, A. N. (2003). Red blood cell magnetophoresis. *Biophysical Journal*, 84(4), 2638–2645.
- Zhu, G., & Trung Nguyen, N. (2010). Particle sorting in microfluidic systems. *Micro and Nanosystemse*, 2(3), 202–216.
- Ziegler-Heitbrock, L., Ancuta, P., Crowe, S., Dalod, M., Grau, V., Hart, D. N., Leenen, P. J. M., Liu, Y. J., MacPherson, G., Randolph, G. J., Scherberich, J., Schmitz, J., Shortman, K., Sozzani, S., Strobl, H., Zembala, M., Austyn, J. M., & Lutz, M. B. (2010). Nomenclature of monocytes and dendritic cells in blood. *Blood*, 116(16), e74–e80.
- Zimmermann, M. B., & Hurrell, R. F. (2007). Nutritional iron deficiency. *The Lancet*, 370(9586), 511–520.
- Zoller, H., & Henninger, B. (2016). Pathogenesis, diagnosis and treatment of hemochromatosis. *Digestive Diseases*, 34(4), 364–373.

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