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Iron deficiency, iron deficiency anaemia and anaemia of inflammation – an overview

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Abstract

Iron is an essential trace element for various cellular proteins and for biological processes in all cells. Severe iron deficiency (ID) impairs haem synthesis, reduces erythropoiesis and causes iron deficiency anaemia (IDA). Iron restriction in anaemia of inflammation is mainly due to retention of iron in macrophages. This condition is known as 'functional iron deficiency'. A review of studies performed in Europe shows that the prevalence of ID and IDA in young children varies by region. It is more common in eastern than western European countries. This overview summarises information on the need for iron supplementation in children, and the current understanding of the regulatory mechanisms of iron homeostasis and iron-restricted erythropoiesis. The causes of anaemia during infection and the usefulness of classical and new indicators to distinguish absolute from functional iron deficiency are discussed.

Key words

inflammation, iron, anaemia

INTRODUCTION

Iron is an essential trace element for various cellular proteins and for biological processes in all cells. As a prosthetic component (e.g. for haem and iron-sulphur clusters), it plays a vital role in the storage and transport of oxygen, the transport of electrons in the respiratory chain, the Krebs cycle, the regulation of gene expression, lipid metabolism, and DNA synthesis and replication. Iron ions are a constituent of respiratory enzymes, such as cytochromes and peroxidases or catalases. In addition to its role in oxygen transport by haemoglobin, iron has been shown to contribute to brain development, nervous and immune system function, and cognitive performance in young children and the elderly. Iron is also essential for the proper maturation and function of oligodendrocytes, which are responsible for myelination in the brain [1]. The animal model shows that severe iron deficiency (ID) has serious and long-term consequences, such as epigenetic changes, brain changes, changes in neurotransmission and myelination. ID also affects the foetal circulation, skeletal muscle and gastrointestinal tract. In infants, ID can result in poor attention and memory, impaired visual and auditory systems. Newborns of severe iron-deficient mothers may have a lower birth weight and more perinatal complications. In the future, they will be at increased risk of high blood pressure, obesity, lipid disorders, attention deficit hyperactivity disorder, and disturbed social and emotional behaviour [2–5].

The WHO estimates that the global prevalence of anaemia in children aged 6–59 months was around 40% in 2019, which means that about 269 million children will be affected, mostly in South-East Asia and Africa. Dietary iron is estimated to account for about 60% of the total global burden of anaemia [6]. Among adolescents, the prevalence of iron deficiency anaemia (IDA) can be as high as 25–30% in countries with a low to medium social development index [7]. There are no precise data on the incidence of ID (without anaemia) in pregnant women; however, it is thought that 30–50% of them may have low ferritin levels at the end of pregnancy [8]. A review of studies performed in Europe shows that the prevalence of IDA in young children varies by region. It is more common in eastern than western European countries and varies according to socio-economic status and the type of milk consumed (i.e. human milk, cow's milk, infant formula). Using the recommended by WHO a cut-off value of 12µg/L for ferritin as a criterion, ID was found in 3–48% of healthy European children aged ≥12 months and in 4–18% of healthy children aged 6–12 months. In most studies, the prevalence of IDA in preschool healthy children in Northern Europe was <5%, compared with 9–50% in Eastern Europe. In Western European children aged 12–36 months, 69% were found to be iron sufficient [9]. In the United States, population iron status was calculated from serum ferritin and soluble transferrin receptor (sTfR) concentrations. Using data from the 2007–2010 National Health and Nutrition Examination Survey, it was found that among children aged 1–5 years, the prevalence of ID and IDA was 7.1% and 1.1%, respectively. The prevalence of ID was higher in children aged 1–2 years than in older children, and was detected in 6.6%-15.2% of toddlers [10, 11]. The authors of this article did not find any data on the prevalence of ID in children in Poland, although it can be assumed that the percentage is similar to that reported by van der Merwe and Eussen for Eastern European countries [9].

Iron homeostasis regulatory mechanisms. Both iron deficiency and iron overload can be harmful. Iron overload can cause a number of complications, including liver damage, heart failure, diabetes mellitus, joint pain and changes in skin colour. They occur in patients with hereditary haemochromatosis, a rare genetic disorder characterized by the accumulation of iron in various organs of the body.

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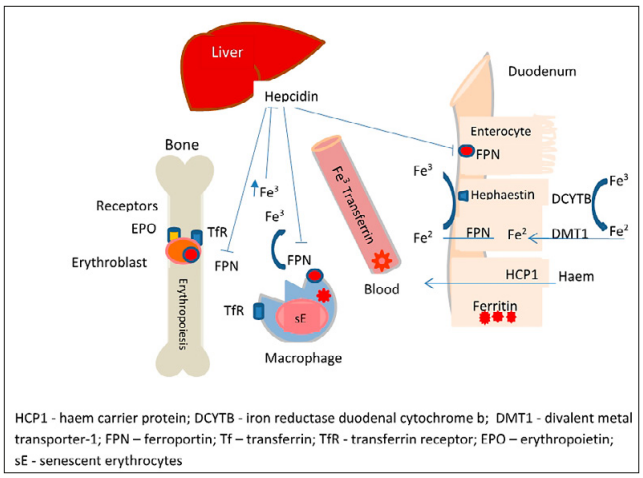
Patients, particularly those with beta thalassemia, may also be at risk of iron overload. Ineffective erythropoiesis, haemolysis of abnormally formed red blood cells, and the need for frequent blood transfusions due to severe anaemia result in an excess of iron. It should be noted that extremely preterm infants may also be at risk of iron overload following frequent blood transfusions. Apart from blood loss, there is no physiologic mechanism for the excretion of excess iron. An excess of free iron is toxic to cells, generating reactive oxygen species (ROS) which are the cause of oxidative stress and ultimately cell death pathways [12]. Free haem is also highly toxic. In order to maintain iron homeostasis, the human organism has a precise control system at many stages, including iron uptake into enterocytes and into other cells and their organelles, iron storage and transport to the extracellular space. The first step in this highly-regulated system is the absorption of dietary iron in the digestive tract (Fig. 1). It is transported directly across the apical membranes of enterocytes by specific haem transporters (haem carrier protein; HCP1). Non-haem iron, however, cannot use this transport. As ferric iron (Fe^{+3}), it must be reduced to ferrous iron (Fe^{+2}) before it can be absorbed, a process carried out by the apical iron reductase duodenal cytochrome b (DCYTB). Ferrous iron is imported into the enterocyte by the apical iron transporter (divalent metal transporter-1; DMT1, also known as NRAMP2 or DCT1). The same protein is responsible for the transport of iron to erythroblasts and other cells. Inside all cells, iron is only present in the form of Fe^{+2} (ionic iron). Within the cells (e.g. duodenal enterocytes, hepatocytes, erythroid cells, macrophages, placenta), ionic iron is transported by ferroportin (FPN, IREG-1, MTP-1), which acts as an iron exporter. Before iron leaves the cells and enters the blood, it must be oxidised back to the Fe^{+3} form, a process in which hephaestin participates. The transition to Fe^{+3} allows iron to bind to the plasma glycoprotein transferrin (Tf), which transports iron in the bloodstream to where it is used or stored. Excess iron in the cell is bound by ferritin. One molecule of this protein has a binding capacity of about 4.500 iron atoms. This is essential to protect cells against the rise in free iron levels, also due to activation of haem oxygenase (HO), which releases it from haem. When needed, iron is released from ferritin by its degradation in cellular lysosomes [13]. Some ferritin accumulated in enterocytes may be lost with the cell, which has a relatively short survival time of 2–5 days. A certain amount of ferritin is found in the blood. Serum ferritin is currently considered the most specific indicator of iron deficiency and iron overload in healthy subjects. It has been established that 1 μg/L of serum ferritin corresponds to 8- 10mg of storage iron [14, 15].



HCP1 - haem carrier protein; DCYTB - iron reductase duodenal cytochrome b; DMT1 - divalent metal transporter-1; FPN – ferroportin; Tf – transferrin; TfR - transferrin receptor; EPO – erythropoietin; sE - senescent erythrocytes

Figure 1. Mechanisms involved in the regulation of systemic iron homeostasis [44]

Iron-restricted erythropoiesis. The iron needed for erythropoiesis is mainly obtained by macrophages of the reticuloendothelial system (e.g. spleen macrophages, Browicz-Kupffer cells in the liver, bone marrow macrophages) from the phagocytosis of senescent red blood cells (RBC) (Fig. 1). Macrophages participate in its storage and in the case of need, release it to the blood. The transfer of iron to the circulation takes place with the participation of ferroportin. The level of iron indirectly controls the production, maintenance and clearance of RBCs. Expression of ferroportin depends on hepcidin, a peptide produced and secreted by hepatocytes. Hepcidin binds to ferroportin in the cell membrane, causing its internalization and degradation. The concentration of hepcidin in the blood determines the amount of iron that enters the circulation. Iron deficiency reduces the concentration of hepcidin and allows iron to be released from the cell (e.g. enterocytes, macrophages) and bound to transferrin. Iron uptake from transferrin is enabled by transferrin binding to the transferrin receptor (TfR) embedded in cell surface membranes. By the internalization of this complex within an endocytic vesicle iron is released from transferrin and transported to the cytosol, and transferrin is recycled back to the cell surface and returned to the bloodstream. ID and hypoxia down-regulate hepcidin synthesis, allowing increased iron absorption and mobilisation from stores.

Other erythroid regulators that act as hepcidin inhibitors include erythropoietin (EPO), sTfR, haemojuvelin, hypoxia-inducible factor 1 (HIF1) and erythroferrone (ERFE). As anaemia and hypoxia develop, EPO synthesis increases and erythroferron inhibits the action of hepcidin. In severe ID, iron regulatory protein 1 (IRP1) reduces the synthesis of erythropoietin in the kidneys which, in turn, reduces the production of red blood cells. This allows iron to be conserved for other cell survival [13, 16].

Iron is required for the synthesis of haemoglobin in the maturing erythroblasts. Erythropoietic stem cells and mature erythrocytes do not synthesise haem or globin. Stem cells contain very few transferrin receptors (TfR), in contrast to more mature erythropoietic cells, which contain large numbers of them in their plasma membranes. When there is insufficient iron, the expression of TfR increases and there is also an increase its soluble form (sTfR) in the blood. As anaemia and hypoxia develop, EPO levels increase and stimulate the division and differentiation of erythroid precursor cells. The increased number of erythroblasts and the limited supply of iron result in a reduction in the haem content per cell. Low heme production reduces globin synthesis. The essential for the translational regulation of globins is heme-regulated inhibitor (HRI), a key protein very important for the survival of erythroid precursors and for appropriate hemoglobin synthesis in iron deficiency. By controlling the globin synthesis their amount is balanced with the amount of intracellular heme available for haemoglobin production. The co-ordination of haem and globin improves erythropoiesis and protects the cell from the cytotoxic effects of each component separately [17].

An increase in iron deficiency to a marginal level will cause a decrease in transferrin saturation, but the haemoglobin level may still be within the normal range. These conditions are also referred to as iron-deficiency erythropoiesis or absolute iron deficiency non-anemic. Severe iron deficiency impairs haem synthesis, reduces erythropoiesis and causes hypoproliferative microcytic anaemia, which is defined as IDA. This is characterized by reduced Hb concentrations, a decrease in the number of red blood cells, and the presence of small erythrocytes with reduced haemoglobin content in a peripheral blood smear. Its common name is microcytic, hypochromic anaemia [18, 19].

Iron deficiency diagnosis and risk factors. In practice, the diagnostic assessment of an anaemic child involves the combination of history, physical examination and laboratory tests. Children with severe IDA have clinical symptoms, such as: pallor, decreased activity, irritability, poor feeding and poor weight gain. Older children may have difficulty concentrating, headaches or tinnitus. Clinical signs suggesting iron deficiency include dry hair and skin, atrophic glossitis, alopecia or koilonychia. Some children with iron deficiency suffer from pica, the compulsive ingestion of non-food items. Many children with mild ID or mild IDA are asymptomatic and their presence is only suggested by risk factors. The causes of iron deficiency can vary depending on the age of the child. In the group of infants up to 6 months of age, it may be due to low maternal iron status, low birth weight, prematurity and lack of iron supplementation [20–22]. For older infants, the causes can include exclusive breastfeeding, introduction of cow's milk before 12 months of age, and excessive cow's milk intake (>500 ml/day). After 12 months of age, ID is more likely to be related to low socio-economic status, low iron in the diet, overweight/obesity, and bleeding. In adolescence, it may also be caused by participation in endurance sports/running [23].

As mentioned above, ID should be suspected in infants born to mothers with ID in the second and third trimester of pregnancy. Dietary iron requirements are significantly increased at this time. Based on a pre-pregnancy weight of 55 kg, it is estimated that an additional 1 g of iron is required, which is an average of about 3.6 mg/day [24, 25]. In developed countries, foetuses are rarely at risk of iron deficiency. During the second and third trimesters of pregnancy, iron is actively transported across the placenta, preferentially to the foetus, even if the mother is iron deficient. At birth, a healthy, full-term infant of an iron-sufficient mother has a high concentration of haemoglobin (140–200g/L) and sufficient iron storage, located mainly in the liver, red blood cells and in macrophages [26]. In the first weeks of life, there is a gradual decrease in the concentration of Hb, the haematocrit and the volume of red blood cells (RBC). Foetal haemoglobin is replaced by adult haemoglobin [27]. Decrease in haematological biomarkers is caused by reduced erythropoietin synthesis due to better oxygenation of the child's tissues. The possibility cannot be ruled out that the rapid decrease in haemoglobin concentration and haematocrit in the first 2 months after birth is also caused by mild haemolysis. Excess iron derived from haemoglobin is immediately stored. In full-term infants born to iron-sufficient mothers, iron reserves reach 75 mg/kg and are mainly found as constituents of haemoglobin and in the form of ferritin. From the third month of life, there is a slow increase in Hb and the number of RBC. The increase in Hb is lower than that of RBC due to the gradual depletion of the child's iron stores. It is assumed that a healthy, exclusively breastfed, full-term infant, whose mother was not iron deficient during pregnancy, will have an adequate iron supply for the first 4–6 months. Infants obtain some iron from breast milk, although the iron content is low (~0.35 mg/L), and its bioavailability is high. After 4–6 months, the iron stores are depleted and the iron obtained from the mother's milk is insufficient to meet the needs of a rapidly growing child with a high level of erythropoietic activity. For this reason, the WHO encourages breastfeeding until the baby is 6 months old, after which time it strongly recommends iron-rich complementary foods, including meat products and/or foods fortified with iron.

The Nutrition Committee of the European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) also strongly recommends exclusive or full breast-feeding for at least 4–6 months. Complementary foods (solid and liquid, other than breast milk or formula) should not be introduced before the age of 4 months, but must be introduced after the age of 6 months [28]. From then on, a child's diet depends more on environment, socio-economic status, eating habits or culture. Meanwhile, between the ages of 6 and 24 months, infants and toddlers experience rapid growth and an increase in iron requirements to support development [25]. Therefore, when designing a child's diet, it is important to ensure that it contains bioavailable iron. Haem iron is better absorbed than non-heme iron, which is found in plants and iron-fortified foods. Both heme and non-heme iron are found in meat, seafood and poultry. Absorption of non-haem iron may be enhanced by vitamin C taken at the same meal. Large amounts of calcium (especially from supplements) and plant substances, such as tannins and phytates, inhibit the absorption of non-haem iron [29, 30]. Given that non-haem iron from plant sources has lower bioavailability and is also more sensitive to the effects of inhibitors, such as phytates, it is reasonable to assume that even healthy individuals in Western countries may be at increased risk of ID. These are vegetarians, especially vegans, but also school-age children, who mainly consume unfortified cereal-based diets. According to the ESPGHAN recommendation, vegan diets should only be used under appropriate medical or dietetic supervision [28]. Parents should be aware of the consequences of not following advice regarding iron needs. According to the European Food Safety Authority Panel on Dietetic Products, Nutrition and Allergies, the estimated average iron requirement for European children is 8 mg/d for infants aged 7–11 months, and 5 mg/d for children aged 1–6 years [31]. This amount of iron should be given in well-chosen nutrients to healthy children who are not at risk of iron deficiency. Additional iron supplementation is recommended for selected groups of children. These include children born prematurely, from multiple pregnancies, too small for gestational age, of mothers with anaemia during pregnancy, or children with perinatal blood loss, as well as children from socio-economically disadvantaged or immigrant families [22, 26].

Premature infants are a special care group at risk of ID and IDA, known as anaemia of prematurity. This is caused by many factors, including shortened RBC survival, low erythropoietin production and decreased erythropoietin response, rapid growth of the child, as well as repeated blood

samples for laboratory tests. There is insufficient evidence to support general iron supplementation of healthy, European infants and toddlers born on time with normal birth weight. Infants who are not breastfed up to 6 months of age should receive an iron fortified infant formula, with an iron content of 4–8 mg/L (0.6–1.2 mg/kg/d). Preterm and other children from the group at risk of iron deficiency should receive iron supplements of 1–3 mg/kg/d for the first 12 months of life, regardless of the diet [32, 33]. According to WHO recommendation, iron supplementation for preventing ID and IDA should be used in countries where the prevalence of anaemia in the group of infants and children is 40% or higher. Daily doses are recommended of 10–12.5 mg of elemental iron (equivalent to 50–62.5 mg of ferrous sulfate heptahydrate, 30–37.5 mg of ferrous fumarate and 83.3–104.2 mg of ferrous gluconate) for 3 consecutive months per year for infants, and children aged 6–23 months, 30 mg for pre-school children (24–59 months), and 30–60 mg of elemental iron daily for schoolchildren (5–12 years) [34].

Anaemia of inflammation. Anaemia of inflammation (AI), also known as inflammatory anaemia, is a type of anaemia that occurs in people with conditions that cause inflammation. These could be infections, autoimmune diseases, chronic kidney disease, gastrointestinal, lung, heart, or metabolic diseases. Features of inflammatory anaemia are also associated with obesity [35, 36]. In this article, the authors focused mainly on ID, IDA and anaemia during infection (AI).

Iron restriction in AI is mainly due to the action of pro-inflammatory proteins that increase hepcidin expression which, in turn, causes iron retention in macrophages and, to a lesser extent, a reduction in iron from the digestive tract. This immune-driven condition is known as 'functional iron deficiency', which, unlike absolute iron deficiency, is caused by an imbalance between iron intake, iron use and iron loss [20, 23 36]. The same people are often affected by both AI and IDA. There are many mechanisms that lead to anaemia, including the excretion of iron by sloughing mucosal cells and blood loss, reduction of the half-life of erythrocyte, reduction in the production of red blood cells due to reduced synthesis of erythropoietin (kidney disease), and reduced response of developing red blood cells to erythropoietin due to cytokines produced by inflammatory cells. It may also be due to increased synthesis of hepcidin [37, 38]. Examples of pathogens that cause anaemia by various mechanisms include parvovirus B19, CMV, Epstein-Barr virus, hepatitis A, B, C and E, SARS-CoV-2, human immunodeficiency virus, T-cell leukaemia, and lymphoma viruses that suppress erythropoiesis [20]. CMV can cause suppression of the erythropoietic system as well as haemolysis.

The SARS-CoV-2 virus appears to mimic the action of hepcidin. It increases the level of ferritin in tissues, reducing the availability of iron needed for erythropoiesis [39]. Parvovirus B19, which causes a disease known as fifth disease, selectively infects erythroid precursors and inhibits erythropoiesis. This virus is particularly dangerous for pregnant women because it is able to invade the erythroid precursor cells of the foetus and cause them to undergo apoptosis [20]. In some cases, the anaemia in the foetus can be so severe that serial transfusions are needed *in utero* and after birth. It is generally known that iron should not be given to patients with viral infections. Some viruses, including SARS-CoV-2, use iron to replicate, causing hypoferraemia and iron-restricted erythropoiesis [20, 39, 40]. The iron is also essential for the growth, virulence and survival of other microbial pathogens, including E. coli, Campylobacter jejuni, Salmonella typhimurium, Myocbacterium bovis BCG and Plasmodium species [41]. As many of them lead to iron loss, the Plasmodium species alter iron metabolism, causing haemolysis and haem release, erythropoiesis disorder, increased deposition of iron in macrophages, and inhibition of dietary iron absorption [42]. Anaemia of inflammation caused by this pathogen is directly related to the geographical burden of infection, mainly in South-East Asia and Africa. Poverty, malnutrition and ID in these region place people at greater risk of viral, bacterial and parasitic infection. In Europe, the common infection that can cause anemia is Helicobacter pylori (H. pylori). Chronic gastritis caused by this bacterium leads to gastric hypochlorhydria and impairs reduction of the dietary iron from the ferric to the ferrous form. H. pylori also needs iron for its growth and competes for it with the host, causing an infection in which it increases hepcidin production which, in turn, decreases the release of iron from macrophages and enterocytes. Other possible mechanisms of anaemia during this infection include haemorrhagic gastritis and active bleeding ulcers. It has been shown that children with H. pylori infection have a lower iron status than healthy age-matched controls, and that successful eradication of H. pylori improves the iron status [43]. Bacterial gastroenteritis, which contributes to ID and IDA, is also caused by enteroinvasive Escherichia and Shigella. Fortunately, improved hygiene has significantly reduced the incidence of prolonged infections caused by these bacteria in Poland.

Haematological and biochemical markers of iron status. Although microbial pathogens exacerbate IDA by competing with the host for iron, and infectious diseases increase iron requirements, iron should be used with extreme caution during infection (if needed). Because AI can coexist with ID, it can sometimes be difficult to decide whether or not the patient needs iron. A variety of biomarkers, both haematological and biochemical, are used to assess iron status. Typical haematological features of IDA are: decreased Hb levels, decreased mean corpuscular Hb concentration (MCHC), decreased mean corpuscular volume (MCV), significant anisocytosis on a peripheral blood smear, elevated the red cell distribution width (RDW), as well as decreased reticulocyte Hb concentration (CHr) and reticulocyte count. Among the most commonly recommended biochemical indicators are: ferritin, total iron binding capacity (TIBC), transferrin saturation (TfSat) and soluble transferrin receptor (sTfR). The basic criterion for diagnosing anaemia is a decrease in Hb concentration below the norm for age and gender. According to the WHO recommendation, population studies on ID and IDA should be conducted for the determination of haemoglobin, ferritin and C-reactive protein or transferrin receptor. The cut-off values of serum ferritin (SF) <12 µg/L to define ID and haemoglobin <110 g/L in combination with SF <12 µg/L to define IDA, should be used [44]. For the majority of the population of adult men, Hb concentration should be not less than 13 g/dL, and for non-pregnant adult women not less than 12 g/dL [45]. In pregnant women, haemoglobin concentration is dependent on the trimester of pregnancy and should be no less than <11.0, <10.5 and <11.0 g/dL in the first,

second and third trimesters, respectively. The cut-off point for haemoglobin concentration to define anaemia in children should be determined by age. According to the WHO, this it determined to be below 11 g/dL in children aged 6 months to 6 years, and below 12 g/dL in children 6–14 years old. Similar ranges of norms are given by other authors: between 6 months and 4 years old – Hb < 11.0 g/dL, 5–11 years old – Hb < 11.5 g/dL, and 12–14 years old – Hb < 12.0 g/dL [46, 47]. In the Polish literature on diagnosing anemia in children, Chaber, on the basis of many publications, gives slightly lower ranges for infants and toddlers: between 6 months and 2 years old – Hb<10.0 g/dL, 2–12 years old – Hb < 11 g/dL, 13–18 years old – 12.0 g/dL [19].

For differentiation of IDA from AI, the haematological features should be combined with the concentration of ferritin. Currently, serum ferritin concentration is considered to be the most efficient and cost-effective test for diagnosing even mild iron deficiency (depletion of iron stores) in subjects without infection. The problem is that the lower limit of normal ferritin concentration, which is generally recommended for the diagnosis of ID, varies widely depending on the source. To define iron deficiency, the WHO recommends ferritin cut-off values of <15 µg/L in apparently healthy children (5 to less than 10 years), adolescents (10 to less than 20 years) and adults, and <12 µg/L in apparently healthy children under 5 years of age [48]. According to Camaschella, in adults, a serum ferritin concentration of less than 30 µg/L should be taken as an indicator of iron deficiency, and less than 10 µg/L, together with haemoglobin, as IDA [49]. For the diagnosis of iron deficiency in patients with inflammatory conditions, such as chronic kidney disease, inflammatory bowel disease or chronic heart failure, a higher ferritin threshold of >100 µg/L or TfSat < 20% is proposed. Ferritin concentration less than 100 µg/L is proposed as an indicator of AI with ID [49, 50]. The recommended cut-off value for serum ferritin in young children to define ID is also between 10–15 µg/L [51, 52]. Mukhtarova *et al.* suggest that in one-year-old children, a serum ferritin threshold of 24–25 µg/L may be a better approach for considering iron supplementation than 10–15 µg/L. Using the lower cut-off can be a loss of time in correcting iron deficiency [52]. In the presence of infection or inflammation, the WHO defines ID as a ferritin concentration of less than 30 µg/L in children under 5 years of age, and less than 70 µg/L in older children and adults [48].

Another commonly used indicator for diagnosing iron status is transferrin saturation (TfSat), the normal range of which is 20–45%. This is the value of serum iron divided by total iron binding capacity (TIBC). TfSat is not only reduced in iron deficiency anaemia, but also in iron distribution disorders, such as infections and chronic inflammation. Therefore, it is not recommended to use it alone, but in combination with ferritin and other markers (Tab. 1). In patients with inflammation, a useful biomarker seems to be reticulocyte haemoglobin content (CHr) [44]. This is an early indicator of the recent functional iron availability and iron-deficient erythropoiesis, less affected by inflammation than transferrin saturation and ferritin. Iron deficiency leads to a decrease CHr, even in the early stages of iron deficiency. It arises within a few days of starting iron therapy and is particularly useful as an early measure of response. In children, a low level of CHr is considered to be a strong predictor of iron deficiency [44]. At this time, there is no standardized cut-off point and different researchers use varying cut-off values [53]. The lowest cut-off point of CHr for the diagnosis of ID found by Ogawaa et al. in the literature, is 27.2 pg [54]. In the authors' institution, the accepted CHr ranges from 22.5–31.8 pg.

The zinc protoporphyrin/haem ratio (ZPP/haem ratio) can also be used as an indicator of the amount of iron supplied to developing erythrocytes and available for haem formation. In the final step of haem biosynthesis, iron forms a chelate with protoporphyrin. During periods of iron deficiency, zinc replaces iron, leading to increased formation of ZPP in erythrocytes. An increased ZPP/haem ratio is one of the first signs of inadequate iron stores and is elevated before anaemia occurs. The normal content of ZPP in erythrocytes is 19–38 µmol/mmol of haem. There are also reference ranges in the paediatric populations [55]. This indicator is now used less frequently and has largely been replaced by the CHr score.

Another indicator that offers hope for the easy, early detection of iron status is soluble transferrin receptors (sTfR). Serum sTfR concentration reflects early functional ID and is higher in the presence of iron deficient erythropoiesis and IDA [56]. Normal adult female and male sTfR concentrations have been found to range from 1.9–4.4 mg/L and 2.2–5.0 mg/L, respectively. The paediatric norm is lower than in healthy adults, ranging from 0.8–3.3 mg/L, and depends on age [57]. Gedfie *et al.*, based on previous publications, suggest that in children without infection, a serum sTfR concentration greater than 3.3 mg/L can be considered the level indicating latent ID [11]. At present, the determination of sTfR is not yet widely available and there is a lack of standardisation in laboratory assessment. However, it may prove very useful in the diagnosis of iron deficiency as it is less influenced by the inflammatory status than other markers.

One of the new parameters that can be used to assess iron resources appears to be hepcidin, which is thought to indicate iron deficiency before anaemia develops. The

Table 1. Haematological and biochemical markers for the diagnosis of iron deficiency (ID) and their suggested interpretation [44, 48]

	Hb	MCV MCHC	ZPP/ haem ratio	CHr	Ferritin [µg/L]	Transf. concent.	TfSat %	sTfR	hepcidin
Normal iron stores	N	N	N	N	>30–60	N	> 20	N	N
Low iron stores	N	N	N	N	15–30	N	> 20 or ↓	N	N or ↓
Iron deficiency without anaemia	N	N or ↓	↑	↓	<15–30	↑	< 20	↑	↓ or ↓↓
Iron deficiency with anaemia	↓	↓	↑	↓	<15–30	↑	< 15	↑	↓↓
Functional ID (anaemia of inflamm. without ID)	↓	↓	↑	↓	N / ↑	N / ↓	Usually < 20	N	↑
Anaemia of inflamm. with ID	↓	↓	↑	↓	<30; <70 up to: age, inflamm.	↓	< 20	N or ↓	N or ↓

N – within the norm, ↓ – below the norm, ↓↓ – very low, ↑ – above the norm, Hb – haemoglobin, MCV – mean corpuscular volume, MCHC – mean corpuscular haemoglobin concentration, CHr – reticulocyte haemoglobin content, ZPP/haem ratio – zinc protoporphyrin/haem ratio, Transf. concent. – transferrin concentration, sTfR – soluble transferin receptor, inflamm. – inflammation

concentration of hepcidin is significantly reduced in patients with iron deficiency, but is increased in patients with severe inflammation. Pagani *et al.* suggest that using hepcidin as an indicator of anaemia can be divided into 2 categories: with high and with low hepcidin [58]. A high level would indicate AI, a low level of hepcidin would indicate IDA. Characteristically, IDA presents with hypochromic and microcytic anaemia. It should be noted that thalassemia carriers may also have hypochromic and microcytic anaemia. Symptoms of beta thalassemia, in the form of severe anaemia, can appear as early as infancy. Ineffective erythropoiesis in these patients contributes to an increase in erythroferrone and a decrease in hepcidin [59]. There are currently no standards for routinely assessing hepcidin levels in adults and children, nor established range of norm.

In conclusion, there is still an insufficient number of well-designed and conducted studies, both epidemiological and clinical, to demonstrate unequivocally the adverse effects of iron deficiency on children's development. The task is not made any easier by the fact that there is still no cheap, easy to determine and independent of other factors, indicator to assess iron resources.

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