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Abstract

Iron deficiency is the most common micronutrient deficiency in the world and represents a major challenge for public health, notably in terms of morbidity and mortality. It remains largely under-diagnosed due to the low level of exploration and the absence of international harmonization for biological tests and thresholds. We performed a systematic review of the literature using PubMed, which allowed us to identify 41 publications within the scope of this review. This analysis shows the benefit of using transferrin saturation in addition to ferritin, in the diagnosis of iron deficiency and even in first-line analysis for patients with chronic inflammatory diseases.

Keywords: Iron deficiency, ferritin, transferrin, saturation.

Introduction

Iron deficiency (ID) is the most common and widespread deficiency in the world[1] and represents a significant challenge for public health[2]. Approximately two billion people, or over 30% of the world population, suffer from anemia principally due to ID[3]. ID, with or without anemia, is associated with fatigue, altered quality of life, and productivity at work[3]. ID is defined by the presence of an insufficient supply of iron to meet the requirements of the organism[2,4]. Despite the high prevalence, including in developed countries[1], ID remains under-diagnosed, due to the absence of international harmonization for diagnostic test and thresholds[2] or the existence of inflammatory disease. When ID is associated with inflammation, the interpretation of serum ferritin usually used to detect it becomes equivocal[5]. This becomes problematic as chronic inflammation is often associated with ID[2]. For example, 37 to 61% of patients with chronic heart failure (CHF), 24 to 85% of patients with chronic kidney disease (CKD) and 13 to 90% of patients with inflammatory bowel disease (IBD) have ID[4]. In these situations, ID can have severe consequences leading to a faster deterioration of clinical signs[4].

According to the assessment report published by the French National Authority for Health (Haute Autorité de Santé, France) in 2011, diagnosis of ID must rely on serum ferritin levels, and in cases of inflammatory pathologies (e.g. CHF, CKD, IBD), must systematically be associated with transferrin saturation (TSAT)[6]. Ironis principally stored in the organism within ferritin and is present in the circulation as a result of release from macrophages and cell lysis. Transferrin is the primary iron transport protein in the circulation. The TSAT (%) is the ratio of serum iron concentration (µmol/L)/total iron-binding capacity (TIBC; µmol/L), after deduction of the weight-adjusted serum concentration of transferrin (g/L).

To refine the mechanisms leading to ID and optimize patient care, two clinical-biological representations have been defined – absolute ID and functional ID – each with different origins, different mechanisms and in particular, different biological profiles. Absolute ID is defined as a decrease in the total iron stores in the body (3 to 4 g for a healthy adult). Absolute ID may be the consequence of insufficient iron intake (older adult, malnutrition or malabsorption) or blood loss[7]. Functional ID, also referred to as iron-restricted erythropoiesis, is due to a defect in iron transport from the storage areas (e.g. macrophages, liver), although the total iron stores in the body are normal or even increased[5]. In this situation, despite a correct level of iron stores, the assimilation of iron by erythroid precursor cells is insufficient[8]. The principal cause of functional ID is inflammation, which leads to increased production of hepatic hepcidin, resulting in dysfunction of ferroportin (an iron transporter), and preventing the export of iron from storage areas[5]. A low concentration of serum ferritin (<100 µg/L) and a low TSAT[5,7] are observed in absolute ID. The functional ID is characterized by a normal or even high serum ferritin concentration and a low TSAT. Therefore, decreased TSAT becomes a particularly performing marker as it does not have similar interpretation limitations as serum ferritin, mainly in inflammatory conditions. This article aims, through a systematic literature review, to assess the benefit of using TSAT as a diagnostic criterion of ID.

Materials and Methods

A first literature analysis was performed using PubMed on January 3rd 2018 using key terms such as "anemia" and "iron deficiency" (Medical Subject Headings (MeSH)), and selecting consensus conferences, guidelines, meta-analyses, practical recommendations, and reviews. The search was limited to documents in English and French published between January 1st 1997 and January 1st 2018. Given the large number of publications identified (N=1165), this first series was abandoned, and the search criteria were refined for the subsequent analyses.

A second review of the literature was performed on January 11th 2018 using key terms such as “anemia” and “iron deficiency” (MeSH), and selecting consensus conferences, recommendations, meta-analyses and practical recommendations. The search was limited to documents in English and French published between January 1st 1997 and January 1st 2018. Then a third search was performed using key terms such as “anemia” and “iron deficiency” (MeSH), restricting to reviews in English and French published between January 1st 2015 and January 1st 2018.

A fourth search was performed using the keywords “anemia", "iron deficiency" (MeSH) and/or "transferrin saturation," selecting consensus conferences, recommendations, meta-analyses, practical recommendations, and reviews. The search was restricted to reviews in English and French with no restriction on the timing of publication.

Finally, a fifth search was performed on April 26th 2018 using the keywords "siderophilin" and "iron deficiency," selecting documents in English and French published between January 1st 1950 and December 31st 1980. This last search sought to take into account the etymological change in the naming of transferrin.

Information on the ferritin threshold values and/or TSAT used in the diagnosis of ID was extracted from each publication. The majority of publications did not mention the dosing methods used nor the nature of the sample taken (serum or plasma).

Results

From searches 2, 3, 4 and 5, a total of 835 references (122 + 216 + 297 + 200) were identified using the defined criteria (***Figure 1***). After verification by two reviewers of titles and/or abstracts, 41 publications were included in the systematic review: six publications from the second literature search, 17 from the third search, 14 from the fourth search and finally, four from the last.

The diagnosis of ID was addressed in some publications (n=36) in the general population[3,8–24] or specific conditions including pregnancy[25], older subjects[5], chronic diseases[4], IBD[2,26–29], CKD[30–36], and CHF[7,37,38]. Other publications (n=5) focused on the levels of one of the markers of ID, either serum ferritin[39–41] or transferrin[42,43].

A deeper analysis showed that for the diagnosis of absolute ID, studies used ferritin alone (10/20) or ferritin with TSAT (10/20), whereas no study used TSAT alone (0/20); the corresponding values for the diagnosis of functional ID are 6/24, 18/24 and 0/24 studies (***Table 1***). Some older studies only addressed the diagnosis of ID (without specifying which type, either absolute or functional) and used ferritin alone (6/21), ferritin with TSAT (13/21) or TSAT alone (3/21). Two of the three studies addressing the diagnosis of ID using TSAT alone were among the oldest of this review[22,43].

Concerning the thresholds used for the diagnosis of ID, these were defined for serum ferritin, in terms of the presence or absence of inflammation as <100 to 300 μg/L or <16 to 100 μg/L, respectively; and for TSAT as <15 to 20% regardless of inflammatory status.

Discussion

ID is a widespread condition often overlooked and under-diagnosed, particularly in patients with chronic inflammation. Based on the review of the literature (835 references, 41 publications analyzed), the diagnosis of ID is mainly based on the concentration of serum ferritin, with variable thresholds in the presence or absence of inflammation, and/or on the TSAT value, with a threshold of <15 to 20% irrespective of inflammatory status.

Several diagnosic methods have been proposed for ID. The analysis of bone marrow (by aspiration or biopsy) is recognized as the “gold standard” for the diagnosis of ID, allowing an estimation of the iron supply in the bone marrow by the Perls staining technique, which reveals hemoseridin[16,29,37,44,45]. This staining method also allows evaluation of the iron stores within macrophages and erythroblasts. For Nielsen *et al.*, the diagnosis is made when <10% of erythroblasts (called sideroblasts) are stained using this method[46]. Nevertheless, this technique, which is costly, non-automated, and uncomfortable for the patient[16,45], is now only used in cases where the diagnosis is uncertain or when results are contradictory [14].

Ferritin, the principal protein for iron storage[40,47], varies in parallel with iron supply in tissues[6]. Serum ferritin concentration, a reflection of the balance between secretion and hepatic clearance, is an indirect method for measuring available iron[47,16]. The World Health Organization has defined the depletion of iron, considered an extreme situation, as serum ferritin levels <15 µg/L in adults and <12 µg/L in children[48]. However, ferritin is also an acute phase protein in inflammation, complicating the interpretation of fluctuations in concentration[4,36]. In infection or inflammation, serum ferritin may be “erroneously normal” meaning the values may be consistent with those in the general population, despite a decrease in total iron supply[2].

The French National Authority for Health has described numerous other situations in which serum ferritin is increased independently of iron supply levels: hepatic and muscular cytolysis, decompensated diabetes, hyperthyroidism, and certain metabolic syndromes[6]. For serum ferritin, it is appropriate to adapt the threshold for the diagnosis of ID to the inflammatory status of patients, particularly in chronic pathologies. Analysis of analytical methods to determine ferritin concentration in France in 2015 through the national quality control report by the Agence Nationale de Sécurité du Médicament et des Produits de Santé (ANSM) showed variability between methods of 16.5% (for 993 laboratories). This variability should also be taken into consideration when interpreting these thresholds. The practical recommendations made in 2017[4] proposed a serum ferritin threshold of 100 µg/L in patients with chronic pathologies such as IBD, CK, and CHF, even if some authors consider the level of ferritin does not give a precise indication of the store of iron that can be released or available for erythropoiesis[33].

TSAT reflects the available quantity of circulating iron and seems to be a better indicator than ferritin of the amount of iron available for bone marrow erythropoiesis and non-erythropoiesis effects. In the blood, iron is mainly bound to a transport protein, transferrin, which can bind one or two atoms of iron[6], and protect the body from iron-induced reactive oxygen species (ROS) production. The synthesis of transferrin increases when the iron supply in the body is low. The iron-transferrin complex binds to cell membranes via the transferrin receptor, after which, the iron is internalized[7] by clathrin-mediated endocytosis. In situations of iron homeostasis, 20% to 45% of the iron binding sites on transferrin are occupied by ferric ions (Fe^{3+}): the TSAT is equal to the ratio of serum iron/total transferrin fixation capacity[40]. An increase in TSAT is associated with the induction of hepcidin expression, whereas a low TSAT has the

opposite effect. Therefore, TSAT reflects the total iron availability in the body (deficiency or overload) and the equilibrium between the release of iron from storage areas and its use by bone marrow for erythropoiesis[40]. Historically, TSAT was most used, before methods for measuring serum ferritin levels became established[41]. The study of Bainton *et al*.[22] in 1964, showed that the available iron supply in the body exhibited a better correlation with TSAT than with the plasma iron concentration in patients presenting with different degrees of ID and suffering from pathologies mimicking ID. TSAT is considered a good indicator of iron reserves in the bone marrow, and a value below 16% confirms iron deficient anemia, either absolute or functionnal[49].

Regardless of the mechanism of ID (absolute or functional), TSAT will be low contrary to serum ferritin (***Table 2***). These diagnostic properties of TSAT are particularly useful, and even superior to those of serum ferritin, in patients affected by chronic inflammatory pathologies. The association of the two markers, ferritin and TSAT, allows an increase in the respective diagnostic performance of both markers[40,7]. Cappellini *et al.*[4] and the French National Authority for Health[6] advocate for the clinical use of the association of the serum ferritin levels with TSAT in the context of chronic inflammatory pathologies. Besides, TSAT provides prognostic information. In non-dialyzed patients with moderate to severe CKD, a reduction in TSAT is associated with an increase in all-cause mortality, independently of serum iron concentration[50]. In patients with CHF, a low TSAT index is independently associated with increased mortality in patients with normal serum iron, whereas a low ferritin concentration is not[49].

The recommendations of the European Society of Cardiology published in 2018[46] stipulate that the serum ferritin concentration and TSAT must be measured and

evaluated together, and ID defined when serum ferritin thresholds below 100 µg/L if TSAT is normal, or from 100 to 299 µg/L when TSAT is below 20%. These threshold values were given in the recommendations of the European Society of Medical Oncology published in 2018[47]. TSAT has certain limitations related to the markers used for its calculation[40]. The iron serum concentration varies according to the iron intake from food, leading to large fluctuations throughout the day of TSAT[30]. Glomerular diseases with proteinuria can lead to a decrease of blood transferrin because of an accelerated leakage of transferrin in the urine, despite a compensatory increase in its production. Excess estrogens (pregnancy, hormonal treatment) can increase the synthesis of transferrin, independently of the iron supply. Hepatocellular lesions affect transferrin concentration. Thus, faced with a suspected ID, the initial outcome should include serum ferritin and measurement of TSAT, in addition to hemoglobin. This type of outcome allows the diagnosis of isolated ID (normal hemoglobin, and ferritin and/or TSAT low), and in case of anemia, to specify the iron levels at the outset[4]. It is interesting to note that these conclusions are in agreement with those obtained for hemochromatosis, or congenital iron overload, where the duration of exposure to a high TSAT is correlated with phenotypic severity, contrary to ferritin, and provides additional information[50].

This literature review has some limitations. Only references from PubMed were extracted using the indexation, MeSH, limiting the number of older articles (before the 1990s). The references were of heterogeneous quality (population, methodology), taking into account the search date went as far back as 1950. Furthermore, many publications we analyzed are review articles where the authors did not give details of specify sensitivity, specificity or clinical performance of the cut-offs stated. As the present publication review spanned more than 50 years, methods of measurement and

calibration for ferritin, iron, and transferrin have evolved over this period. The evolution of methods (e.g. turbidimetric, nephelometric and immunochemistry for ferritin radioimmunoassays) constitutes a limitation for comparing the thresholds.

In conclusion, the diagnosis of ID can be complicated to perform in the clinic. The most potent biological parameters to diagnose ID are the measure of serum ferritin levels and a calculation of TSAT. The measure of TSAT appears to be a better reflection of the amount of available iron for bone marrow erythropoiesis than ferritinemia. As TSAT is less prone to fluctuations than ferrtin, particularly in patients suffering from chronic inflammatory diseases, TSAT seems most relevant to diagnose ID.

Conflict of Interest Statement: Patrice Cacoub has received consulting and lecturing fees from Abbvie, AstraZeneca, Bristol-Myers Squibb, Gilead, GlaxoSmithKline, Janssen, Merck Sharp & Dohme, Servier, and Vifor. Katell Peoc'h has received consulting and lecturing fees from France, Fresenius, Kabi, Masimo, Octapharma, Werfen, Vifor, Siemens Healthineers, Abbott, Brahms, Diasorin and research grants from MSD Avenir and Fujirebio.

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Figure 1. Flow chart of the selection of publications for the literature review.

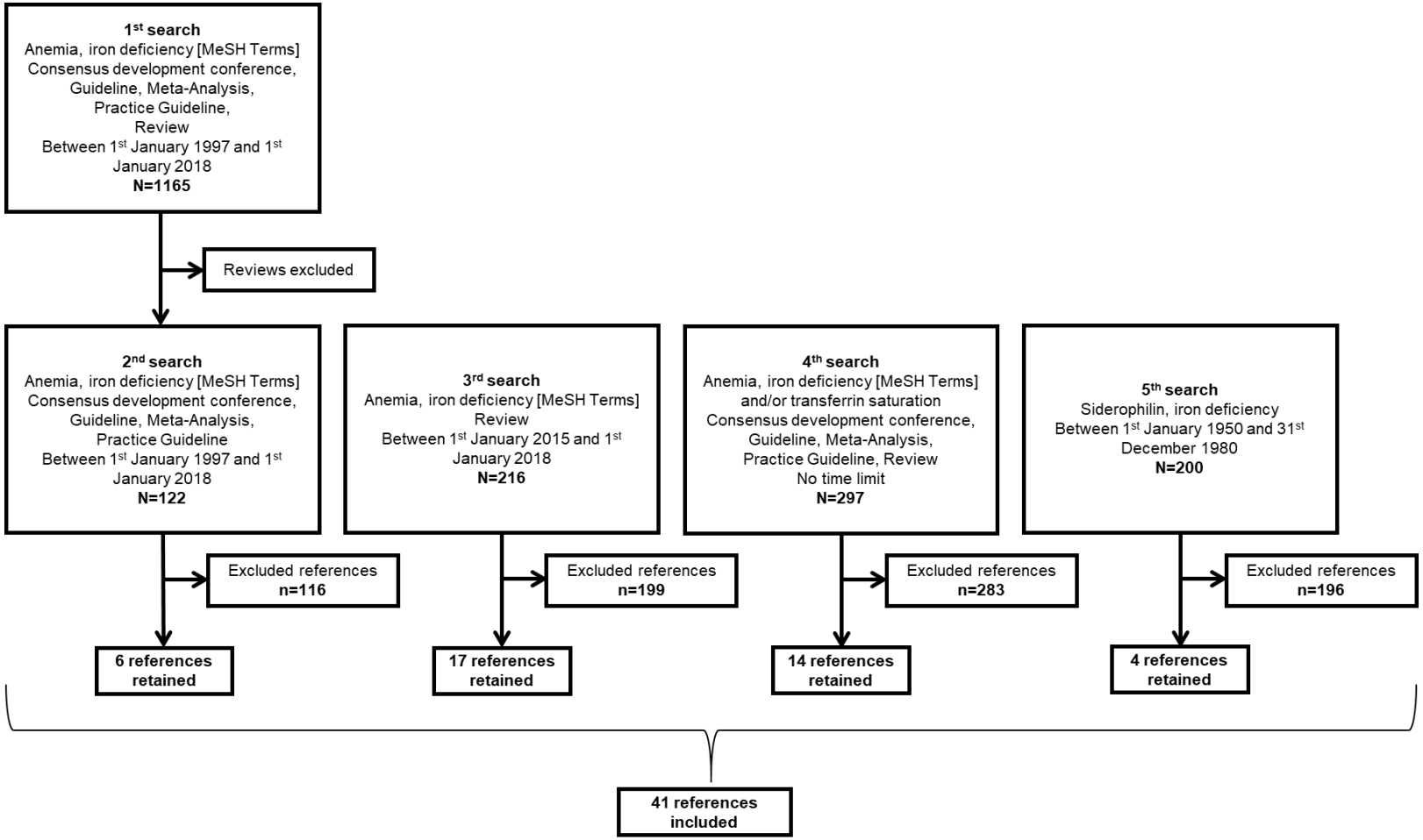

Table 1. Principal publications assessing the diagnostic thresholds of ferritin and TSAT for iron deficiency.

Author, year	Publication type	Population studied	Pathological context	Serum ferritin	TSAT
Dignass et al., 2018[2]	European consensus	IBD	Iron deficiency Absence of inflammation Presence of inflammation Chronic illness anemia	 <30 µg/L <100 µg/L >100 µg/L	 - - <20%
Bou-Fakhredin et al., 2017[26]	Review	IBD	Iron deficiency	<100 µg/L Si 100 – 300 µg/L	- TSAT <20 %
Cappellini et al., 2017[4]	International recommendations	Chronic diseases (CHF, CKF, IBD)	Iron deficiency independent of anemia	<100 µg/L 100-300 µg/L	TSAT <20% TSAT <20%
Daru et al., 2017[39]	Review	Patients with or without inflammation	Iron deficiency	<30 µg/L	-
Gaweda, 2017[34]	Review	CKD	Iron deficiency in ND Iron deficiency in HD	<100 ng/mL <200 ng/mL	<20 % <20 %
Goodnough et al., 2017[41]	Review	CHF	Iron deficiency	<300 ng/mL	<20 %
Konishi et al., 2017[37]	Review	CHF	Absolute iron deficiency	<100 µg/L <300 µg/L	- <20 %
Pfeiffer et al., 2017[16]	Review	Patients with or without inflammation	Iron-deficient anemia Chronic diseases anemia	Decreased Normal or increased	Decreased Decreased
Peyrin-Biroulet et al., 2015[3]	Review	Healthy subject and patient (adults; children)	Absolute iron deficiency Functional iron deficiency	<100 µg/L >100 µg/L	<20% <20%
Auerbach et al., 2016[12]	Review	-	Iron deficiency	<15 – 25 ng/mL	<20 %
Bahrainwala et al., 2016[30]	Review	CKD	Iron-deficient anemia, pre-dialysis or PD Iron-deficient anemia, HD	<100 ng/mL <200 ng/mL	 <20 %
Elsayed et al., 2016[42]	Review	-	Iron deficiency	-	<20 %
Lopez et al., 2016[13]	Review	-	Iron deficiency Iron deficiency with inflammation Iron deficiency in ND Iron deficiency in HD	<15 µg/L <50 µg/L <100 µg/L <200 µg/L	<16 % <20 %
Nairz et al., 2016[11]	Review	-	Iron-deficient anemia	<30 ng/mL	<16 %
Nielsen et al., 2016[24]	Review	With or without chronic diseases	Iron deficiency without inflammation Iron deficiency with inflammation	<30 µg/L <100 µg/L	<16 % <20%
Stein et al., 2016[27]	Review	Gastrointestinal and renal diseases	Iron deficiency without inflammation Iron deficiency with inflammation	<30 ng/mL <100 ng/mL	<20 % <20 %
Suchdev et al., 2016[23]	Epidemiological study	Patients with iron deficiency	Iron deficiency, with or without inflammation	<30 µg/L	-
Archer et al., 2015[14]	Review	Patients with iron deficiency	Iron deficiency	<12 µg/L	-

Author, year	Publication type	Population studied	Pathological context	Serum ferritin	TSAT
			Iron deficiency in ND Iron deficiency in HD	<100 µg/L <200 µg/L	- -

Table 1 (cont.). Principal publications assessing the diagnostic thresholds of ferritin and TSAT for iron deficiency.

Author, year	Publication type	Population studied	Pathological context	Serum ferritin	TSAT
Camaschella, 2015[15]	Review	-	Iron deficiency	<30 µg/L	<16 %
			Functional iron deficiency	Normal	Low - normal
			Iron deficient anemia	<10 µg/L	<16 %
			Iron deficiency with inflammation	<100 µg/L	
			Iron deficiency, CHF or CKF	<300 µg/L	<30 %
Le Petitcorps et al., 2015[5]	Review	Older subject	Functional iron deficiency	Normal or increased	<20 %
			Absolute iron deficiency	<50 – 100 µg/L	<20 %
Rukuni et al., 2015[25]	Review	Pregnant women	Iron deficiency	<15 µg/L	-
Cohen-Solal et al., 2014[7]	French expert cardiologists	CHF	Absolute iron deficiency	<100 µg/L	-
			Functional iron deficiency	100 – 299 µg/L	<20 %
Shander et al., 2014[17]	Review	Anemic patients	Iron deficient anemia	<30 ng/mL	<20 %
			Inflammatory anemia	<200 ng/mL	<15 %
Thomas et al., 2013[8]	English recommendations	CKD	Iron deficiency in ND	<100 µg/L	-
			Iron deficiency in HD	<200 µg/L	-
Goddard et al., 2011[9]	English recommendations	-	Iron deficiency without inflammatory pathology	12 – 15 µg/L	-
			Iron deficiency with inflammatory pathology	<50 µg/L	-
Muñoz et al., 2011[18]	Review	According to inflammatory status	No inflammation: Absolute iron deficiency	<15 - 30 µg/L	-
			With inflammation:		
			- Absolute iron deficiency	<100 ng/mL	<20 %
			- Functional iron deficiency	>100 ng/mL	<20 %
Oustamanolakis et al., 2011[28]	Review	IBD	Iron deficiency without inflammation	<30 µg/L	<16 %
			Iron deficiency with inflammation	<100 µg/L	<16 %
			Chronic diseases anemia	>100 µg/L	<16 %

Table 1 (cont.). Principal publications assessing the diagnostic thresholds of ferritin and TSAT for iron deficiency.

Author, year	Publication type	Population studied	Pathological context	Serum ferritin	TSAT
Bermejo et al., 2009[29]	Review	Digestive diseases	Iron deficiency, without inflammation Iron deficiency, with inflammation	<30 ng/L <100 ng/mL	 <20 %
Handelman et al., 2008[20]	Review	According to the anemia type	Iron deficiency Iron deficiency in CKF	<12 µg/L <200 µg/L	<10 % <20 – 50 %
Madore et al., 2008[35]	Recommendations	CKD	Iron deficiency in ND without ESA Iron deficiency in ND and DP receiving ESA Iron deficiency in HD receiving ESA	<100 ng/mL <100 ng/mL <200 ng/mL	<20 % <20 % <20 %
Zimmermann, 2008[19]	Review	Healthy adults	Iron deficiency	<15 µg/L	<15 %
Goodnough et al., 2007[36]	Review	Patients receiving EPO	Iron deficiency in anemic patient Iron deficiency in dialyzed patient	<30 – 40 µg/L <200 µg/L <400 µg/L	- - <20 %
Agarwal, 2006[31]	Review	CKD	Iron deficiency	<100 ng/mL	<20 %
Wish, 2006[21]	Review	CKD	Absolute iron deficiency Functional iron deficiency	<100 ng/mL Normal or increased	<20 % <20 %
CDC, 1998[10]	American recommendations	Children (>6 months), adolescents, pregnant women of childbearing potential	Iron deficiency	<15 µg/L	<16 %
Bickford, 2002[32]	Review	CKD	Iron deficiency	<100 ng/mL	<20 %
Macdougall, 1994[33]	Review	Patients with EPO	Iron deficiency	<100 µg/L	<20 %
Koerper et al., 1977[43]	Threshold analysis	Healthy children	-	-	<16 %
Jacobs et al., 1972[41]	Review e	Healthy subjects, iron deficient anemia and iron overload	Iron deficiency	<10 ng/mL	<16 %
Mazza et al., 1978[40]	Clinical validation of serum iron and TSAT	Patients having had a myelogram	Iron deficiency	<18 ng/mL	<20 %
Bainton et al., 1964[22]	Clinical validation of iron deficiency criteria	Patients with iron deficiency or diseases mimicking iron deficiency	Iron deficient anemia	-	<16 %

TSAT, transferrin saturation index; ESA, erythropoiesis-stimulating agent; CKD, chronic kidney disease; PD, peritoneal dialysis; EPO, erythropoietin; CHF, chronic heart failure; CKF, chronic kidney failure; IBD, inflammatory bowel disease; HD, hemodialysis; ND; non-dialyzed.

Table 2. Variations in the transferrin saturation index based on total iron availability

	Serum iron concentration	**Transferrin**	**Transferrin saturation**	**Ferritinemia**
Absolute iron deficiency	Decreased	Increased	Decreased	Decreased
Functional iron deficiency	Normal or increased	Decreased	Decreased	Normal or increased