European Journal of Pediatrics (2019) 178:41–49
https://doi.org/10.1007/s00431-018-3257-0

ORIGINAL ARTICLE

The usefulness of reticulocyte haemoglobin content, serum transferrin receptor and the sTfR-ferritin index to identify iron deficiency in healthy children aged 1–16 years

María A. Vázquez-López[1] · Encarnación López-Ruzafa[1] · Mercedes Ibáñez-Alcalde[2] · Manuel Martín-González[1] · Antonio Bonillo-Perales[1] · Francisco Lendínez-Molinos[1]

Received: 29 January 2018 / Revised: 21 June 2018 / Accepted: 17 September 2018 / Published online: 27 September 2018

Abstract

This cross-sectional study, conducted on a population-based representative sample, evaluates the usefulness of reticulocyte haemoglobin content (CHr), serum transferrin receptor (sTfR) and sTfR/log ferritin (sTfR-F index) to recognise iron deficiency (ID) without anaemia, provides specific cut-off points for age and gender, and proposes a new definition of ID. A total of 1239 healthy children and adolescents aged 1–16 years were included. Complete blood count, iron biomarkers, erythropoietin, C-reactive protein, CHr, sTfR, and sTfR-F index were determined. ROC curves were obtained and sensitivity, specificity, predictive values, likelihood ratios, and accuracy for each specific cut-off points were calculated. Seventy-three had ID without anaemia. Area under the curve for sTfR-F index, sTfR and CHr were 0.97 (CI95% 0.95–0.99), 0.87 (CI95% 0.82–0.92) and 0.68 (CI95% 0.61–0.74), respectively. The following cut-off points defined ID: sTfR-F Index > 1.5 (1–5 years and 12–16 years boys) and > 1.4 (6–11 years and 12–16 years girls); sTfR (mg/L) > 1.9 (1–5 years), > 1.8 (6–11 years), > 1.75 (12–16 years girls) and > 1.95 (12–16 years boys); and CHr (pg) < 27 (1–5 years) and < 28.5 (6–16 years).

Conclusions: CHr, sTfR and the sTfR-F index are useful parameters to discriminate ID without anaemia in children and adolescents, and specific cut-off values have been established. The combination of these new markers offers an alternative definition of ID with suitable discriminatory power.

What is Known:
- *In adults, reticulocyte haemoglobin content (CHr), serum transferrin receptor (sTfR) and sTfR/log ferritin index (sTfR-F index) have been evaluated and recognised as reliable indicators of iron deficiency (ID).*
- *Clinical manifestations of ID may be present in stages prior to anaemia, and the diagnosis of ID without anaemia continues to pose problems.*

What is New:
- *CHr, sTfR and the sTfR-F index are useful parameters in diagnosis of ID in childhood and adolescence when anaemia is not present.*
- *We propose a new strategy for the diagnosis of ID in childhood and adolescence, based on the combination of these measures, which offer greater discriminatory power than the classical parameters.*

Keywords Iron deficiency · Reticulocyte haemoglobin content · Serum transferrin receptor · sTfR-F index

Communicated by Peter de Winter

✉ Encarnación López-Ruzafa
e.lopezruzafa@gmail.com

María A. Vázquez-López
mavazquezl59@gmail.com

Mercedes Ibáñez-Alcalde
mmibanezalcalde@gmail.com

Manuel Martín-González
manuel.martin.gonzalez.sspa@juntadeandalucia.es

Antonio Bonillo-Perales
antonio.bonillo.sspa@juntadeandalucia.es

Francisco Lendínez-Molinos
lendimol@gmail.com

[1] Department of Pediatrics, Torrecárdenas Hospital, Hermandad de Donantes de Sangre s/n, 04009 Almería, Spain

[2] Department of Pediatrics, Poniente Hospital, Avenida Almerimar s/n, 04007 El Ejido, Spain

Abbreviations

ACD	Anaemia of chronic disease
AUC	Area under the curve
CHr	reticulocyte haemoglobin content
CI	confidence interval
CRP	C-reactive protein
EP	erythrocyte protoporphyrin
ID	iron deficiency
IDA	iron-deficiency anaemia
IDE	iron-deficient erythropoiesis
ISD	iron stores depletion
LR-	negative likelihood ratio
LR+	positive likelihood ratio
MCV	mean corpuscular volume
MDD	maximum diagnostic discrimination
NPV	negative predictive value
P	percentile
PPV	positive predictive value
ROC	receiver operating characteristics
sEPO	serum erythropoietin
sTfR	serum transferrin receptor
sTfR-F	sTfR/log ferritin
TIBC	total iron binding capacity
TS	transferrin saturation

Introduction

Childhood and adolescence are periods of increased iron requirements and also of possible iron deficiency (ID) [1, 2, 5, 14, 19, 22, 36, 48]. This condition is the result of a continuous, progressive negative balance of iron. Fundamentally, iron balance is regulated by the rate of erythropoiesis and by the size of iron stores in the body. In childhood and adolescence, the changes involved in growth affect both iron status and erythropoiesis.

In order to determine iron status, various haematologic and biochemical parameters must be considered [21, 48], and in this respect, new diagnostic markers have been incorporated in recent decades. In adults, reticulocyte haemoglobin content (CHr), serum transferrin receptor (sTfR) and sTfR/log ferritin index (sTfR-F index) have been evaluated and recognised as reliable indicators of ID [18, 40], and it has been suggested they should be used in a diagnostic plot to discriminate functional ID, iron-deficiency anaemia (IDA) and anaemia of chronic disease (ACD), and to predict the response to treatment [39].

Erythrocytes have a long lifespan, and therefore erythrocyte indices are late indicators of ID. However, CHr is considered a real-time marker of functional ID, as reticulocytes only remain in blood for 1–2 days [39]. This parameter can be measured in modern haematology analysers and provide the paediatrician with valuable information [28]. Although Brugnara et al. [6], in 1999, and subsequent studies have corroborated the value of CHr in the diagnosis of ID in children [13, 24, 42, 43], little research has been conducted to define normal values for healthy children and adolescents.

sTfR is the soluble form of the transmembrane transferrin receptor that is present in every cell of the body. Its value is proportional to the mass of cellular TfR, which depends primarily on the intracellular requirements of iron and on erythropoietic activity [4, 33, 35]. This parameter provides information about the functional iron compartment and is a useful measure for diagnosing ID [32, 33, 38, 49]. It has advantages over other iron parameters (sTfR is unaffected by inflammation), but the non-standardisation of the measure is a major disadvantage. Despite this reservation, normal values have been published for healthy children and adolescents [12, 29, 37, 45, 46], as have cut-off values indicative of ID in adults [32, 33] and children [3].

The sTfR-F index was proposed by Punnonen et al. [32] as a useful marker of the iron supply available for erythropoiesis. It offers information about iron stores and functional pools and could enhance the diagnostic efficacy of sTfR or that of serum ferritin alone [4, 32, 34, 35].

In a population-based study in which healthy children and adolescents were included, reference values of the above markers were defined, according to strict selection criteria [17, 23, 45, 46]. The next step would be to evaluate the diagnostic utility of these markers in order to establish specific cut-off values for ID which could be incorporated into the diagnostic battery as an alternative to the classical parameters or at least to propose their use in clinical situations in which the latter are less useful. Most studies of the usefulness of CHr, sTfR and the sTfR-F index have been performed with adult populations [18, 32, 38]. Very few studies have been conducted with children and adolescents to establish equivalent cut-off values for these parameters in order to discriminate ID when anaemia is not present.

Accordingly, the goals of the present study are to evaluate the usefulness of CHr, sTfR and the sTfR-F index as a means of recognising ID without anaemia in healthy children and adolescents, to obtain specific cut-off points by age and gender, and to propose an alternative definition of ID based on these new measures.

Methods

Design

Descriptive study on evaluation of diagnostic test.

Subjects

Participants were selected from a cross-sectional study conducted on a population-based representative sample of healthy

children aged 1–16 years in the city of Almería (Spain), from 2007 to 2009.

Selection was performed by multistage probability sampling method described in previous reports [23, 45]. The Healthcare District of Almería provided a list of 5453 children aged 12–47 months. Assuming an ID prevalence of 20%, a 95% confidence interval (CI) and 5% precision, a minimum sample size of 330 children were required. Four hundred forty-four were selected, 19% refused and finally 360 were included. The Municipal Education Office provided a list of the 17,934 children aged 4–11 years from primary schools and 9823 adolescents aged 12–16 years from secondary schools. Assuming an ID prevalence of 10%, a 95% CI and 5% precision, 551 children aged 4–16 years were required. Then, 1323 were invited to participate, 17% refused and 1099 were included.

The criteria for exclusion were (a) haematological or systemic diseases that could affect the parameters analysed, (b) iron therapy in the previous 3 months and (c) the presence of previous infectious or febrile disease or C-reactive protein (CRP) value > 0.5 mg/dl [39].

Survey

The survey was addressed to all of parents, as described in a previous study [15]. A detailed medical history and a complete physical exploration were performed to all selected subjects.

Parameters measured

Haemoglobin, red cell indices, reticulocyte haemoglobin content (CHr) and reticulocytes were performed in ADVIA-120 counter (Siemens Healthcare Diagnostics, NY); erythrocyte protoporphyrin (EP) determined by fluorometric assay [10]; serum transferrin, serum ferritin and CRP determined by the immunoturbidimetric method using "Tina-quant-Transferrin," "Tina-quant Ferritin" and CRPLX kits obtained from Roche Diagnostics GmbH (Boehringer Mannheim) respectively; serum iron determined by colorimetric assay using the Fe kit obtained from Roche Diagnostics GmbH (Boehringer Mannheim); total iron binding capacity (TIBC), calculated as 1 mg transferrin/dl × 1.27 = 1 μg IBC/dl; transferrin saturation (TS), calculated as serum iron/TIBC × 100; serum erythropoietin (sEPO) determined by enzyme immunoassay using the Quantikine IVD-Human Erythropoietin kit (R&D Systems, Minneapolis); sTfR, determined by immunoturbidimetric assay using the Quantex sTfR kit (Biokit SA, Barcelona) and the sTfR-F Index, calculated as sTfR/log ferritin [32].

Iron status was classified normal, iron store depletion (ISD), iron-deficient erythropoiesis (IDE) and iron-deficiency anaemia (IDA). ISD was defined as serum ferritin (ng/ml) < 10 (1–5 years) and < 12 (6–16 years); IDE was defined as ≥ 2 parameters altered: serum ferritin (as indicated above), TS (%) < 10 (1–2 years), < 12 (3–5 years) and < 14 (6–16 years), EP (μg/dl erythrocytes) > 80 (1–2 years) and > 70 (3–16 years); IDA was defined as IDE + haemoglobin (g/dl) < 11 (1–5 years), < 11.5 (6–11 years and 12–16 years girls) and < 12 (12–16 years boys). ISD or IDE were considered as ID without anaemia. In cases of isolated microcytosis, thalassaemia traits (α or ß) were excluded.

Statistical analysis

SPSS 20.0 software for Windows and Epidat 4.0 for the sample calculation were used. The results obtained are expressed as mean ± SD and 95% confidence interval (CI). The qualitative variables are expressed as percentages. Student's *t* test was applied to compare the means, and the ANOVA test was used for comparisons of more than two groups. When the results were significant, differences between groups were identified using the Bonferroni post hoc test. In all cases, a *p* value < 0.05 was considered significant.

To evaluate the clinical usefulness of CHr, sTfR and the sTfR-F index to recognise ID without anaemia, subjects with IDA were excluded. Receiver operating characteristics (ROC) curves were used, obtaining the area under the curve (AUC) and the corresponding 95% CI. For each group, classified by age and gender (1–5 years, 6–11 years, 12–16 years girls and 12–16 years boys), the maximum diagnostic discrimination (MDD) cut-off point was calculated, corresponding to the highest (sensitivity+specificity)/2 value. Sensitivity, specificity, positive and negative predictive values (PPV, NPV), positive and negative likelihood ratios (LR+, LR−), accuracy and the respective 95% CI were calculated for each MDD value. They were also calculated for the $P_{2.5}/P_5$ value for CHr and for the $P_{95}/P_{97.5}$ value for sTfR and the sTfR-F index, when the MDD value excluded more than 5% of the subjects. As CHr, sTfR and the sTfR-F index were assumed to be confirmatory parameters of ID, the ideal cut-off points were selected according to the highest specificity (> 95%) and LR+ (> 5–10) values, as long as the sensitivity was > 25%. The cut-off points selected were used to propose an alternative definition of ID, and finally, the overall prevalence was calculated.

Results

A total of 1342 children aged 1–16 years were enrolled in the study. Of these, the following were excluded: 70 who had C-reactive protein > 0.5 mg/dl, 9 with β or α-thalassaemia trait and 24 due to the absence of relevant analytical results. Finally, 1239 children were included, of whom 620 (50%) were female. By age, the distribution was as follows: 460 (37.1%) aged 1–5 years, 49 (39.6%) aged 6–11 years

and 288 (25.9%) aged 12–16 years. By iron status, 84 (6.8%) had some degree of ID; of these, 13 (1%) had IDA. The prevalence of ID varied according to age. Thus, in the group aged 1–5 years, 24 (5.2%) had ID without anaemia and 9 (2%) had IDA. In those aged 6–11 years, only 16 (3.3%) had ID without anaemia, and among the adolescents (aged 12–16 years), 31 (10.8%) had ID without anaemia and 4 (1.4%) had IDA.

Table 1 shows the mean values obtained for the analytical parameters, according to iron status. Erythropoietic activity was normal in all subjects. Concerning the study parameters, as the ID progressed, CHr values decreased significantly and sTfR and the sTfR-F index increased ($p < 0.0001$).

Figure 1 shows the ROC curves obtained to assess the discriminatory value for ID without anaemia of the classical markers of iron status (mean corpuscular volume (MCV), serum ferritin, TS and EP) and that of the proposed indicators (CHr, sTfR and the sTfR-F index), for the total group (excluding children with IDA). Thus, on the ROC curve shown on the left, serum ferritin is outstanding, with AUC: 0.99 (95%CI 0.99–1). This parameter is clearly preferable to MCV, TS and EP. Among the proposed new parameters (shown in the ROC curve on the right), the sTfR-F index performed best, with a discriminatory power similar to that of ferritin, followed by sTfR with an AUC of 0.87(95%CI 0.82–0.92), which was still better than the classical parameters. The AUC for CHr was similar to that found for MCV, TS and EP. Maximum diagnostic discrimination (MDD) cut-off points were obtained for CHr, sTfR and the sTfR-F index (Fig. 1). MDD values for CHr and sTfR did not meet the established validity criteria, and so these were rejected; instead, CHr P_5 and sTfR P_{95} cut-off points are proposed (Fig. 1: shaded columns).

Table 2 shows the mean values for CHr, sTfR and the sTfR-F index in children with normal iron status for age and gender. CHr values increased with age. The highest values for sTfR were found in the group of adolescents. For the sTfR-F index, the lowest values were obtained for the children aged 6–11 years. Gender only had a significant influence in the adolescents, for sTfR and the sTfR-F index, but not for CHr. This finding enabled specific cut-off points for age and gender to be established. Tables 3 and 4 show the sensitivity, specificity, predictive values, likelihood ratios and accuracy obtained for all ages and gender groups, for each cut-off value. Those presenting greatest overall utility, which are shaded in the tables, were selected. Accordingly, CHr values lower than the shaded cut-off values, and higher ones for sTfR and the sTfR-F index, would be suggestive of ID.

With these new parameters, an alternative definition of ID without anaemia could thus be proposed: (a) ISD: ferritin (ng/ml) < 10 (1–5 years) and < 12 (6–16 years); (b) IDE: sTfR-F Index > 1.5 (1–5 years and 12–16 years boys) and > 1.4 (6–11 years and 12–16 years girls), or ≥ 2 parameters: serum ferritin (as indicated above); sTfR (mg/L) > 1.9(1–5 years), > 1.8 (6–11 years), > 1.75 (12–16 years girls) and > 1.95 (12–16 years boys); CHr (pg) < 27 (1–5 years) and < 28.5 (6–16 years).

Under these new criteria, 117 children (9.4%) were considered ID.

Discussion

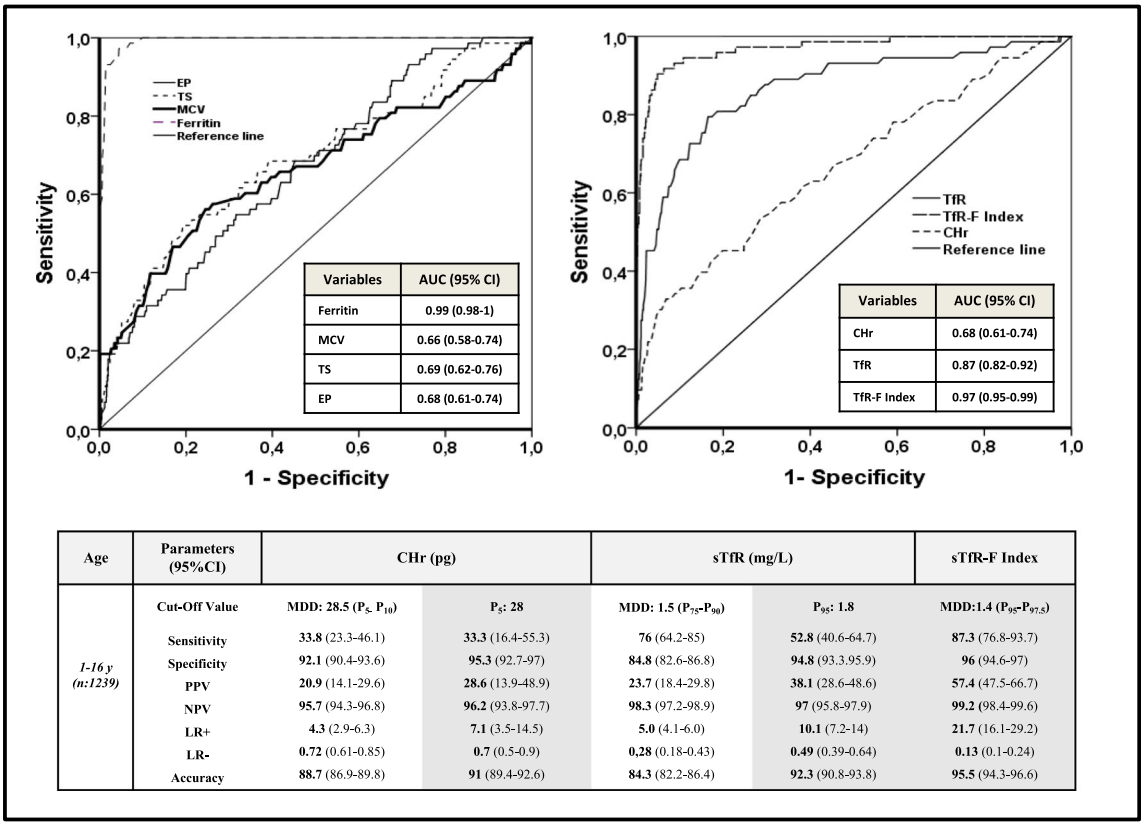
ID is still a health problem among children and adolescents. Its related clinical manifestations may be present in stages

Table 1 Mean values of iron parameters and erythropoietin according to iron status in healthy children aged 1–16 years

Iron status	Normal *n* 1153		ID without anaemia *n* 70		IDA *n* 13		*ANOVA p*
	Mean ± SD	*(95% CI)*	*Mean ± SD*	*95% CI*	*Mean ± SD*	*95% CI*	
Hb (g/dl)	13.3 ± 0.9	(13.2–13.3)	13 ± 0.84†	(12.8–13.2)	10.5 ± 0.7‡	(10.1–10.9)	0.0001
MCV (fl)	77.9 ± 3.5	(77.7–78.1)	75.2 ± 5.3*	(73.9–76.4)	67.5 ± 5.2‡	(64.3–70.6)	0.0001
TS (%)	24.4 ± 11.9	(23.7–25.1)	17.5 ± 10.5*	(15–19.9)	6.5 ± 2.5£¶	(4.9–8)	0.0001
Ferritin (ng/ml)	30.9 ± 14.8	(30–31.7)	10 ± 2.2*	(9.5–10.5)	6.7 ± 3.3£	(4.7–8.7)	0.0001
EP (μg/dl Hm)	29.2 ± 16.9	(28.2–30.2)	41.6 ± 21.5*	(36.6–46.6)	91.7 ± 63‡	(53.3–130)	0.0001
CHr (pg)	31.1 ± 1.73	(31–31.2)	29.6 ± 2.5*	(29–30.2)	24.5 ± 30‡	(22.7–26.3)	0.0001
sTfR (mg/L)	1.24 ± 0.29	(1.22–1.25)	1.79 ± 0.4*	(1.7–1.88)	2.77 ± 0.40‡	(2.52–3)	0.0001
sTfR-F Index	0.87 ± 0.25	(0.86–0.89)	1.83 ± 0.5*	(1.72–1.95)	4.03 ± 1.61‡	(3.05–5)	0.0001
EPO (mU/L)	8.8 ± 3.6	(8.5–9.0)	11.4 ± 5.7*	(9.7–13.2)	22.4 ± 11.3‡	(13.6–31.1)	0.0001

ID iron deficiency, *IDA* iron deficiency anaemia, *Hb* haemoglobin, *MCV* mean corpuscular volume, *TS* transferrin saturation, *EP* erythrocyte protoporphyrin, *CHr* haemoglobin content of reticulocytes, *sTfR* soluble transferrin receptor, *sTfR-F Index* sTfR/log Ferritin, *CI* confidence interval, *SD* standard deviation

ANOVA: Post hoc (Bonferroni test): †$p < 0.01$ (ID without anaemia/normal); *$p < 0.0001$ (ID without anaemia/normal); ‡$p < 0.0001$ (IDA/normal and IDA/ID without anaemia); £$p < 0.0001$ (IDA/normal); $p < 0.006$ (IDA/ID without anaemia)



Age	Parameters (95%CI)	CHr (pg)		sTfR (mg/L)		sTfR-F Index
1-16 y (n:1239)	Cut-Off Value	MDD: 28.5 (P_5- P_{10})	P_5: 28	MDD: 1.5 (P_{75}-P_{90})	P_{95}: 1.8	MDD:1.4 (P_{95}-$P_{97.5}$)
	Sensitivity	**33.8** (23.3-46.1)	**33.3** (16.4-55.3)	**76** (64.2-85)	**52.8** (40.6-64.7)	**87.3** (76.8-93.7)
	Specificity	**92.1** (90.4-93.6)	**95.3** (92.7-97)	**84.8** (82.6-86.8)	**94.8** (93.3.95.9)	**96** (94.6-97)
	PPV	**20.9** (14.1-29.6)	**28.6** (13.9-48.9)	**23.7** (18.4-29.8)	**38.1** (28.6-48.6)	**57.4** (47.5-66.7)
	NPV	**95.7** (94.3-96.8)	**96.2** (93.8-97.7)	**98.3** (97.2-98.9)	**97** (95.8-97.9)	**99.2** (98.4-99.6)
	LR+	**4.3** (2.9-6.3)	**7.1** (3.5-14.5)	**5.0** (4.1-6.0)	**10.1** (7.2-14)	**21.7** (16.1-29.2)
	LR-	**0.72** (0.61-0.85)	**0.7** (0.5-0.9)	**0,28** (0.18-0.43)	**0.49** (0.39-0.64)	**0.13** (0.1-0.24)
	Accuracy	**88.7** (86.9-89.8)	**91** (89.4-92.6)	**84.3** (82.2-86.4)	**92.3** (90.8-93.8)	**95.5** (94.3-96.6)

Fig. 1 Receiver operating characteristic curves for iron parameters and cut-off points of CHr, TfR and the TfR-F Index for discriminating ID without anaemia in healthy children aged 1–16 years

prior to anaemia [8, 22, 25, 36], and the diagnosis of ID without anaemia continues to pose problems [31, 48].

In the period 2007–2009, a cross-sectional study was conducted in Almería (Spain) in healthy children aged 1–16 years,

Table 2 Mean values of CHr, TfR and the TfR-F Index in healthy children aged 1–16 years with normal iron status according to age and gender

Variables	1–5 years *n* 427 (222 girls) Mean ± SD (95% CI)	6–11 years *n* 475 (238 girls) Mean ± SD (95% CI)	12–16 years *n* 253 (109 girls) Mean ± SD (95%CI)	ANOVA
CHr (pg)				
Total	30.4 ± 1.8 (30.2–31.6)†	31.4 ± 1.6 (31.3–31.6)	31.5 ± 1.3 (31.4–31.7)	0.0001
Girls	30.5 ± 1.8 (30.3–30.8)	31.6 ± 1.5 (31.4–31.7)	31.5 ± 1.2 (31.3–31.8)	
Boys	30.2 ± 1.8 (30.0–30.5)	31.6 ± 1.5 (31.1–31.6)	31.5 ± 1.4 (31.3–31.8)	
p	0.1	0.16	0.94	
sTfR (mg/L)				
Total	1.24 ± 0.29 (1.22–1.27)*‡	1.19 ± 0.26 (1.17–1.22)£	1.32 ± 0.30 (1.28–1.36)	0.0001
Girls	1.22 ± 0.30 (1.18–1.26)	1.17 ± 0.26 (1.14–1.21)	1.23 ± 0.26 (1.18–1.28)	
Boys	1.26 ± 0.29 (1.22–1.30)	1.21 ± 0.27 (1.18–1.25)	1.40 ± 0.32 (1.34–1-44)	
p	0.16	0.16	0.0001	
sTfR-F Index				
Total	0.91 ± 0.27 (0.89–0.94)¶	0.83 ± 0.22 (0.81–0.85)£	0.90 ± 0.25 (0.87–0.93)	0.0001
Girls	0.89 ± 0.27 (0.86–0.93)	0.80 ± 0.21 (0.78–0.83)	0.86 ± 0.22 80.82–0.90)	
Boys	0.93 ± 0.27 (0.89–0.97)	0.84 ± 0.21 (0.82–0.88)	0.94 ± 0.29 (0.88–0.98)	
p	0.19	0.45	0.017	

CHr reticulocyte haemoglobin content, *sTfR* soluble transferrin receptor, *sTfR-F Index* sTfR/log ferritin, *SD* standard deviation, *CI* confidence interval, *p* signification level

ANOVA: Post-hoc (Bonferroni test): †$p < 0.0001$ (1–5 years/6–11 years and 1–5 years/12–16 years); *$p < 0.02$ (1–5 years/6–11); ‡$p < 0.002$ (1–5 years/12–16 years); £$p < 0.0001$ (6–11 years/12–16 years); ¶$p < 0.0001$ (1–5 years/6–11 years)

Table 3 Sensitivity, specificity, predictive values, likelihood ratios and accuracy of CHr, sTfR and the sTfR-F Index to discriminating ID without anaemia in healthy children aged 1–11 years

Age group	Parameters (95%CI)	CHr (pg)		sTfR (mg/L)		sTfR-F Index
1–5 years (*n* 451)	Cut-off value	MDD 29 (P_{10}–P_{25})	P_5: 27	MDD 1.6 (P_{90})	$P_{97.5}$ 1.9	MDD 1.5 (P_{95}–$P_{97.5}$)
	Sensitivity	50 (29.6–70.3)	33.3 (16.4–55.3)	79.2 (57.3–92.0)	66.7 (44.7–83.6)	84 (63.1–94.7)
	Specificity	78.9 (74.7–82.6)	95.3 (92.7–97)	88.7 (85.3–91.5)	97.6 (95.6.98.8)	96.5 (94.1–97.9)
	PPV	11.8 (6.5–20)	28.6 (13.9–48.9)	28.3 (18.3–40.9)	61.5 (40.7–79.1)	58.3 (38.3–71.7)
	NPV	96.5 (93.9–98.1)	96.2 (93.8–97.7)	98.7 (96.8–99.5)	98.1 (96.2–99.1)	99 (97.4–99.7)
	LR+	2.4 (1.5–3.7)	7.1 (3.5–14.5)	7.0 (5–9.8)	28.5 (14.5–55.9)	23.8 (14.1–40.3)
	LR−	0.6 (0.4–0.9)	0.7 (0.5–0.9)	0.2 (0.1–0.5)	0.34 (0.2–0.6)	0.16 (0.1–0.4)
	Accuracy	77.4 (75.4–81.3)	92 (89.5–94.5)	99.2 (85.2–91.2)	96 (94.2–97.8)	97.5 (96.1–98.9)
6–11 years (*n* 491)	Cut-off value	MDD 28.5 (P_5)	$P_{2.5}$ 28	MDD 1.6 (P_{90}- P_{95})	P_{95}–$P_{97.5}$ 1.8	MDD 1.4 ($P_{>97.5}$)
	Sensitivity	25 (8.3–52.6)	18.7 (5–46.3)	81.2 (53.7–95)	68.7 (41.5–87.9)	93.7 (62.7–99.7)
	Specificity	95.1 (92.7–96.8)	96.8 (94.7–98.1)	93.5 (90.7–95.4)	96.6 (94.5–98)	98.3 (96.6–99.2)
	PPV	14.8 (4.8–34.6)	16.7 (4.4–42.2)	29.5 (17.2–45.4)	40.7 (23–61)	65.2 (42.8–82.8)
	NPV	97.4 (95.4–98.6)	97.2 (96.2–98.5)	99.3 (97.9–99.8)	98.9 (97.3–99.6)	99.8 (98.6–100)
	LR+	5.2 (2–13.2)	5.9 (1.9–18.5)	12.4 (8.2–18.8)	20.4 (11.4–36-6)	55.6 (27.7–112)
	LR−	0.79 (0.59–1.05)	0.84 (0.66–1.06)	0.2 (0.1–0.5)	0.32 (0.15–0.67)	0.06 (0.01–0.4)
	Accuracy	92.9 (90.6–95.2)	94.3 (92.2–96.3)	93.1 (90.9–95.3)	95.7 (93.9–97.5)	98.2 (97–99.4)

MDD maximum diagnostic discrimination point, *PPV* positive predictive value, *NPV* negative predictive value, *LR+* Likelihood ratio positive; *LR−* Likelihood ratio negative; *95% CI* 95% confidence interval, *P* percentile

to analyse the prevalence of ID and to obtain reference values for new diagnostic parameters such as CHr, sTfR and the sTfR-F index [17, 23, 45, 46]. The main aim of the present study is to analyse the usefulness of these parameters for detecting ID without anaemia.

A total of 1239 healthy subjects were included in the study. According to the classical ID criteria [21], IDA was present in 1% and ID without anaemia in 5.8% of the study population. Both values are in the expected range for developed countries [5, 14, 19, 48]. In agreement with previous studies [3, 6, 20, 26, 42, 47], our analysis showed that CHr decreased significantly as ID progressed, while sTfR and the sTfR-F index increased (Table 1).

Several studies have reported the clinical usefulness of CHr, sTfR and the sTfR-F index in the diagnosis of IDA in children [7, 13, 16, 42, 47] and adults [9, 18, 44], with AUC values usually ≥ 0.80. However, opinions differ as to which of these markers is the best ID indicator. The suitability of these parameters for discriminating states of ID without anaemia has been studied very little [3, 30], and reliable values have not been established. Indeed, some studies have concluded that CHr and TfR have only limited value for detecting iron depletion and that they are not good indicators of early stages of ID [11, 42].

In the present study, we analyse the diagnostic utility of these parameters to discriminate ID without anaemia (Fig. 1). The ROC curve obtained for the sTfR-F index had an AUC of 0.97(95%CI 0.95–0.99), which was very similar to that of ferritin. Therefore, the index is a highly sensitive and specific measure in the diagnosis of ID. Moreover, sTfR alone presents greater diagnostic value than the classical parameters, with an AUC of 0.87 (95%CI 0.82–0.92). These results are comparable to those reported in previous studies [3, 9, 18, 20, 27, 30, 47]. However, CHr alone was not found to be sufficiently indicative in early stages of ID (AUC 0.68, 95%CI 0.61–0.74) [7, 9, 13, 27, 44], although it was comparable to the classical parameters. This finding could reflect the fact that subjects with IDA were excluded from this study, and that CHr only begins to present alterations when the production of haemoglobin is compromised. Nevertheless, we believe that CHr can offer added value, by improving diagnostic efficiency, if it is used together with other markers of the functional iron compartment.

We have previously reported that CHr, sTfR and the sTfR-F index vary significantly according to age and gender [17, 23, 45, 46], as shown in Table 2. For this reason, we tested the validity of these measures for different cut-off values, in order to choose the most suitable. The MDD points, as shown in Tables 3 and 4, were not always considered reliable, as the values obtained were close to those offered by the P_{10} of CHr and the P_{90} of sTfR-F and the index, assuming that 10% of subjects with supposedly normal iron status would be classified as ID. In these cases, we chose specific cut-off values that would more safely confirm the ID status and we propose different discriminatory points to define ID according to age and gender. We assume that lower values of CHr and higher ones of sTfR and the sTfR-F index, with respect to the cut-off values obtained (shaded columns), would be suggestive of ID.

Table 4 Sensitivity, specificity, predictive values, likelihood ratios and accuracy of CHr, sTfR and the sTfR-F Index to discriminating ID without anaemia in healthy adolescents aged 12–16 years

Ages group	Parameters (95%CI)	CHr (pg)	sTfR (mg/L)		sTfR-F Index	
Girls 12–16 years (*n* 132)	Cut-off value	MDD 30 (P_{10})	$P_{2.5}$ 28.5	MDD 1.46 (P_{75}–P_{90})	P_{95}: 1.75	MDD 1.25(P_{90}–P_{95})	$P_{97.5}$ 1.4
	Sensitivity	43.5 (23.9–65.1)	39.1 (20.5–61.2)	76.2 (52.4–91)	30 (12.8–54.3)	85.7 (62.6–96.2)	80.9 (57.4–93.7)
	Specificity	91.1 (83.8–95.4)	100 (95.8–100)	92.7 (74–89)	95.4 (89.2–98.3)	95.4 (89.2–98.3)	99.1 (94.3–99.9)
	PPV	50 (27.8–72.1)	100 (62.9–100)	45.7 (29.2–63.1)	54.5 (24.6–81.8)	77.3 (55.8–91.7)	94.5 (70.6–99.7)
	PPN	88.7 (81.1–93.6)	88.9 (81.7–93.5)	94.8 (87.7–98)	88.2 (80.7–93.2)	97 (91.5–99.3)	96.4 (90.6–98.9)
	LR+	4.9 (2.3–10.2)	Infinite	4.4 (2.7–7.1)	6.6 (2.2–19.6)	18.8 (7.9–45.2)	88 (12.5–626)
	LR-	0.6 (0.4–0.9)	0.6 (0.4–0.8)	0.29 (0.13–0.62)	0.73 (0.55–0.98)	0.15 (0.05–0.43)	0.2 (0.08–0.46)
	Accuracy	83 (76.7–89.3)	89.6 (84.5–94.7)	81 (75.1–88.3)	84.7 (78.5–90.9)	98.8 (89.7–97.9)	96.1 (92.7–99.4)
Boys 12–16 years (*n* 152)	Cut-off value	MDD: 30 (P_{10})	$P_{2.5}$–P_5 28.5	MDD 1.65(P_{75}–P_{90})	P_{95}: 1.95	MDD 1.4 (P_{90}–P_{95})	P_{95}–$P_{97.5}$: 1.5
	Sensitivity	50 (22.3–77.7)	25 (6.7–57.1)	90 (54.1–99.5)	45.4 (18.1–75.4)	90.1 (57.1–99.5)	90.9 (57.1–99.5)
	Specificity	83.7 (76.3–89.2)	97.1 (92.4–99.1)	81.5 (73.7–87.4)	94.8 (89.1–97.7)	91.8 (85.4–95.6)	96.2 (91.1–98.26
	PPV	20.7 (8.7–40.2)	42.8 (11.8–79.7)	26.5 (13.5–44.6)	41.7 (16.5–71.4)	47.6 (26.4–69.7)	66.7 (38.7–87)
	PPN	95.2 (89.3–98)	93.8 (88.3–96.9)	99.1 (94.3–99.9)	95.5 (90–98.1)	99.2 (94.9–99.9)	99.2 (95.1–99.9)
	LR+	3.1 (1.5–6)	8.8 (2.2–34.9)	4.9 (3.2–7.3)	8.7 (3.3–22.9)	11.1 (6.1–20.1)	24.3 (10.1–58.7)
	LR–	0.6 (0.34–1)	0.77 (0.55–1.1)	0.12 (0.02–0.79)	0.57 (0.33–0.9)	0.1 (0.01–0.64)	0.09 (0.01–0.61)
	Accuracy	81 (74.8–87.2)	91.5 (87.1–95.9)	82.8 (76.7–88.9)	91 (86.4–95.6)	91.7 (87.2–96.2)	95.9 (92.7–99.1)

MDD maximum diagnostic discrimination point, *PPV* positive predictive value, *NPV* negative predictive value; *LR+* Likelihood ratio positive, *LR–* Likelihood ratio negative, *95% CI* 95% confidence interval, *P* percentile

According to the validity obtained for these new parameters, even in early stages of ID, a new definition based on the combination of these measures together with ferritin and haemoglobin can be offered. This new approach makes it possible to determine the full spectrum of body iron status in the different compartments (iron stores and functional iron), not only as an alternative to the classical definition, but also as a useful indicator for situations (such as inflammation or chronic disease) in which the classical parameters are less useful.

Thus, ID without anaemia would be defined as (a) *iron-store depletion*: low ferritin; (b) *iron-deficient erythropoiesis*: high sTfR-F index (which would reflect situations with high sTfR and low ferritin but above the diagnostic cut-off value, and also conditions in which there is iron-store depletion but no impact on sTfR); (c) or the combination of ≥ 2 parameters among low ferritin, low CHr and high sTfR.

When these criteria are applied, the overall prevalence of ID increases.

The benefits and contributions of this study arise from the following methodological considerations: (a) it is based on an extensive and representative population; (b) it was conducted in a city in south-eastern Spain with a broad representation of social classes and ethnic groups, and therefore the results obtained can be extrapolated to other contexts, making it comparable, for example, to other Spanish and European cities; (c) it includes a wide range of paediatric ages; (d) strict selection criteria were applied to define ID; (e) the parameters we propose are of proven utility in the diagnosis of ID and have been included in clinical guidelines for the differential diagnosis of anaemia in adults; (f) we also include the measure of serum erythropoietin as an indirect marker of erythropoietic activity (capable of altering sTfR values); (g) in choosing the diagnostic cut-off points, the likelihood ratio was calculated because it is independent of the prevalence of the disease, and because very few reports have been made of its inclusion in validity studies of diagnostic tests.

This study presents some limitations. Firstly, there is no "gold standard" with which to define ID. Although iron in bone marrow is considered to be the best indicator, this too has its limitations. Moreover, it was not applicable in our study. Secondly, the non-standardisation of the method used to determine sTfR might be viewed as a limitation with respect to generalising the results. Nevertheless, until a reference reagent, developed by the WHO [41] becomes available, the immunoturbidimetric method is currently the most commonly used. Therefore, the results and cut-off values we obtain can be extrapolated and applied to a population of similar characteristics, when a similar methodology is used.

In conclusion, this study highlights the usefulness of CHr, sTfR and the sTfR-F index as a means of discriminating ID without anaemia. We define specific cut-off values by age and gender and offer a new strategy for the diagnosis of ID in

childhood and adolescence, based on the combination of the above measures, which offer greater discriminatory power than the classical parameters. Further studies are required to confirm these results, when sTfR measurement becomes standardised.

Acknowledgments We want to thank all the children and parents involved in the research for their disinterested collaboration.

Authors' contributions María A. Vázquez-López contributed to conception and design, analysis and interpretation of the data and writing of the manuscript. Encarnación López-Ruzafa contributed to conception and design, analysis and interpretation of the data and writing of the manuscript; Mercedes Ibáñez-Alcalde and Manuel Martín-González contributed to the acquisition of data; Antonio Bonillo-Perales and Francisco Lendinez-Molinos critically revised the manuscript and contributed with their final suggestions. All the authors approved the final manuscript for publication.

Compliance with ethical standards

Ethical approval The Ethics and Research Committee of Torrecárdenas Hospital (Almería) approved the study.

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Conflict of interest The authors declare that they have no conflict of interest.

Informed consent Informed written consent was obtained from parents or legal guardians as well as from the participants themselves if they were > 12 years.

References

1. Ahankari AS, Myles PR, Fogarty AW, Dixit JV, Tata LJ (2017) Prevalence of iron-deficiency anaemia and risk factors in 1010 adolescent girls from rural Maharashtra, India: a cross-sectional survey. Public Health 142:159–166. https://doi.org/10.1016/j.puhe.2016.07.010
2. Akbari M, Moosazadeh M, Tabrizi R, Khatibi SR, Khodadost M, Heydari ST, Tahami AN, Lankarani KB (2017) Estimation of iron deficiency anemia in Iranian children and adolescents : a systematic review and meta-analysis. Hematology 22:231–239
3. Angeles Vázquez López MA, Molinos FL, Leyva-Carmona ML et al (2006) Serum transferrin receptor in children: usefulness for determinating the nature of anemia in infection. J Pediatr Hematol Oncol 28:809–815. https://doi.org/10.1097/MPH.0b013e31802d751a
4. Beguin Y (2003) Soluble transferrin receptor for the evaluation of erithropoiesis and iron status. Clin Chim Acta 329:9–22
5. Brotanek JM, Gosz J, Weitzman M, Flores G (2007) Iron deficiency in early childhood in the United States: risk factors and racial/ethnic disparities. Pediatrics 120:568–575. https://doi.org/10.1542/peds.2007-0572
6. Brugnara C, Zurakowski D, DiCanzio J, Boyd T, Platt O (1999) Reticulocyte hemoglobin content to diagnose iron deficiency in children. J Am Med Assoc 281:2225–2230
7. Brugnara C, Schiller B, Moran J (2006) Reticulocyte hemoglobin equivalent (Ret He) and assessment of iron-deficient states. Clin Lab Haematol 28:303–308. https://doi.org/10.1111/j.1365-2257.2006.00812.x
8. Carter RC, Jacobson JL, Burden MJ, Armony-Sivan R, Dodge NC, Angelilli ML, Lozoff B, Jacobson SW (2010) Iron deficiency anemia and cognitive function in infancy. Pediatrics 126:e427–e434. https://doi.org/10.1542/peds.2009-2097
9. Castel R, Tax MG, Droogendijk J et al (2012) The transferrin / log (ferritin) ratio : a new tool for the diagnosis of iron deficiency anemia. Clin Chem Lab Med 50:1343–1349. https://doi.org/10.1515/cclm-2011-0594
10. Chisolm JJ, Brown DH (1975) Micro-scale photofluorometric determination of "free erythrocyte porphirin" (Protoporphyrin IX). Clin Chem 21:1669–1682
11. Choi JW (2005) Sensitivity, specificity, and predictive value of serum soluble transferrin receptor at different stages of iron deficiency. Ann Clin Lab Sci 35:435–439
12. Choi JW, Pai SH, Im MW, Kim SK (1999) Change in transferrin receptor concentrations with age. Clin Chem 45:1562–1563
13. Davidkova S, Prestidge TD, Reed PW, Kara T, Wong W, Prestidge C (2016) Comparison of reticulocyte hemoglobin equivalent with traditional markers of iron and erythropoiesis in pediatric dialysis. Pediatr Nephrol 31:819–826. https://doi.org/10.1007/s00467-015-3284-2
14. Ferrari M, Mistura L, Patterson E et al (2011) Evaluation of iron status in European adolescents through biochemical iron indicators: the HELENA study. Eur J Clin Nutr 65:340–349. https://doi.org/10.1038/ejcn.2010.279
15. García-García E, Vázquez-López MA, García-Fuentes E, Rodríguez-Sánchez FI, Muñoz FJ, Bonillo-Perales A, Soriguer F (2012) Iodine intake and prevalence of thyroid autoimmunity and autoimmune thyroiditis in children and adolescents aged between 1 and 16 years. Eur J Endocrinol 167:387–392. https://doi.org/10.1530/EJE-12-0267
16. Grant FKE, Suchdev PS, Flores-Ayala R, Cole CR, Ramakrishnan U, Ruth LJ, Martorell R (2012) Correcting for inflammation changes estimates of iron deficiency among rural Kenyan preschool children. J Nutr 142:105–111. https://doi.org/10.3945/jn.111.146316
17. Ibáñez-Alcalde M, Vázquez-López MÁ, Ruíz-Sánchez AM et al (2017) Reference values of reticulocyte hemoglobin content in healthy adolescents. J Pediatr Hematol Oncol 40:298–303. https://doi.org/10.1097/MPH.0000000000001119
18. Infusino I, Braga F, Dolci A, Panteghini M (2012) Soluble transferrin receptor (sTfR) and sTfR/log ferritin index for the diagnosis of iron-deficiency anemia: a meta-analysis. Am J Clin Pathol 138:642–649. https://doi.org/10.1309/AJCP16NTXZLZFAIB
19. Levi M, Rosselli M, Simonetti M, Brignoli O, Cancian M, Masotti A, Pegoraro V, Cataldo N, Heiman F, Chelo M, Cricelli I, Cricelli C, Lapi F (2016) Epidemiology of iron deficiency anaemia in four European countries: a population-based study in primary care. Eur J Haematol 97:583–593. https://doi.org/10.1111/ejh.12776
20. Lin X-M, Ji C-Y, Liu W-J, Long Z, Shen XY (2006) Levels of serum transferrin receptor and its response to Fe-supplement in Fe-deficient children. Br J Nutr 96:1134–1139. https://doi.org/10.1017/BJN20061954
21. Looker A, Dallman P, Carroll M, Gunter EW, Johnson CL (1997) Prevalence of iron deficiency in the United States. J Am Med Assoc 277:973–976. https://doi.org/10.1001/jama.277.12.973
22. Lopez A, Cacoub P, Macdougall IC, Peyrin-Biroulet L (2016) Iron deficiency anaemia. Lancet 387:907–916. https://doi.org/10.1016/S0140-6736(15)60865-0
23. López-Ruzafa E, Vázquez-López MA, Lendinez-Molinos F, Poveda-González J, Galera-Martínez R, Bonillo-Perales A, Martín-González M (2016) Reference values of reticulocyte hemoglobin content and their relation with other indicators of iron status

in healthy children. J Pediatr Hematol Oncol 38:e207–e212. https://doi.org/10.1097/MPH.0000000000000639
24. Lorenz L, Arand J, Büchner K, Wacker-Gussmann A, Peter A, Poets CF, Franz AR (2015) Reticulocyte haemoglobin content as a marker of iron deficiency. Arch Dis Child Fetal Neonatal Ed 100: F198–F202. https://doi.org/10.1136/archdischild-2014-306076
25. Lozoff B, Castillo M, Clark KM, Smith JB, Sturza J (2014) Iron supplementation in infancy contributes to more adaptive behavior at 10 years of age. J Nutr 144:838–845. https://doi.org/10.3945/jn.113.182048.adulthood
26. Malope BI, MacPhail AP, Alberts M, Hiss DC (2001) The ratio of serum transferrin receptor and serum ferritin in the diagnosis of iron status. Br J Haematol 115:84–89
27. Markovic M, Majkic-Singh N, Ignjatovic S, Singh S (2007) Reticulocyte haemoglobin content vs . soluble transferrin receptor and ferritin index in iron deficiency anaemia accompanied with inflammation. Int J Lab Hematol 29:341–346. https://doi.org/10.1111/j.1365-2257.2006.00875.x
28. Mast AE, Blinder MA, Lu Q, Flax S, Dietzen DJ (2002) Clinical utility of the reticulocyte hemoglobin content in the diagnosis of iron deficiency. Blood 99:1489–1491. https://doi.org/10.1182/blood.V99.4.1489
29. Ooi CL, Lepage N, Nieuwenhuys E, Sharma AP, Filler G (2009) Pediatric reference intervals for soluble transferrin receptor and transferrin receptor-ferritin index. World J Pediatr 5:122–126. https://doi.org/10.1007/s12519-009-0024-3
30. Phiri KS, Calis JCJ, Siyasiya A, Bates I, Brabin B, van Hensbroek MB (2009) New cut-off values for ferritin and soluble transferrin receptor for the assessment of iron deficiency in children in a high infection pressure area. J Clin Pathol 62:1103–1106. https://doi.org/10.1136/jcp.2009.066498
31. Powers JM, Daniel CL, McCavit TL, Buchanan GR (2016) Deficiencies in the management of iron deficiency anemia during childhood. Pediatr Blood Cancer 63:743–745. https://doi.org/10.1002/pbc
32. Punnonen K, Irjala K, Rajamaki A (1997) Serum transferrin receptor and its ratio to serum ferritin in the diagnosis of iron deficiency. Blood 89:1052–1057
33. Skikne B, Flowers C, Cook J (1990) Serum transferrin receptor. A quantitative measure of tissue iron deficiency. Blood 75:1870–1876
34. Skikne BS, Punnonen K, Caldron PH, Bennett MT, Rehu M, Gasior GH, Chamberlin JS, Sullivan LA, Bray KR, Southwick PC (2011) Improved differential diagnosis of anemia of chronic disease and iron deficiency anemia: a prospective multicenter evaluation of soluble transferrin receptor and the sTfR/log ferritin index. Am J Hematol 86:923–927. https://doi.org/10.1002/ajh.22108
35. Speeckaert MM, Speeckaert R, Delanghe J (2011) Biological and clinical aspects of soluble transferrin receptor. Crit Rev Clin Lab Sci 47:213–228
36. Subramaniam G, Girish M (2015) Iron deficiency anemia in children. Indian J Pediatr 82:558–564. https://doi.org/10.1007/s12098-014-1643-9
37. Suominen P, Virtanen A, Lehtonen-Veromaa M, Heinonen OJ, Salmi TT, Alanen M, Möttönen T, Rajamäki A, Irjala K (2001) Regression-based reference limits for serum transferrin receptor in children 6 months to 16 years of age. Clin Chem 47:935–937
38. Thomas C, Thomas L (2002) Biochemical markers and hematologic indices in the diagnosis of functional iron deficiency. Clin Chem 48:1066–1076
39. Thomas C, Kirschbaum A, Boehm D, Thomas L (2006) The diagnostic plot. A concept for identifying different states of iron deficiency and monitoring the response to epoetin therapy. Med Oncol 23:23–36
40. Thomas DW, Hinchliffe RF, Briggs C, Macdougall IC, Littlewood T, Cavill I, British Committee for Standards in Haematology (2013) Guideline for the laboratory diagnosis of functional iron deficiency. Br J Haematol 161:639–648. https://doi.org/10.1111/bjh.12311
41. Thorpe SJ, Heath A, Sharp G, Cook J, Ellis R, Worwood M (2010) A WHO reference reagent for the serum transferrin receptor (sTfR): international collaborative study to evaluate a recombinant soluble transferrin receptor preparation. Clin Chem Lab Med 48:815–820. https://doi.org/10.1515/CCLM.2010.167
42. Uijterschout L, Domellöf M, Vloemans J, Vos R, Hudig C, Bubbers S, Verbruggen S, Veldhorst M, de Leeuw T, Teunisse PP, van Goudoever JB, Brus F (2014) The value of Ret-Hb and sTfR in the diagnosis of iron depletion in healthy, young children. Eur J Clin Nutr 68:882–886. https://doi.org/10.1038/ejcn.2014.70
43. Ullrich C, Wu A, Armsby C, Rieber S, Wingerter S, Brugnara C, Shapiro D, Bernstein H (2005) Screening healthy infants for iron deficiency using reticulocyte hemoglobin content. JAMA 294:924–930. https://doi.org/10.1001/jama.294.8.924
44. Urrechaga E, Borque L, Escanero JF (2013) Erythrocyte and reticulocyte indices in the assessment of erythropoiesis activity and iron availability. Int J Lab Hematol 35:144–149. https://doi.org/10.1111/ijlh.12013
45. Vázquez-López MA, Ibáñez-Alcalde M, Lendínez-Molinos F, Ruíz-Sánchez AM, Galera-Martínez R, García-García E, Parrón-Carreño T, Bonillo-Perales A (2015) Reference values of serum transferrin receptor and sTfR/log ferritin index in healthy adolescents. J Pediatr Hematol Oncol 37:274–280
46. Vázquez-López MA, López-Ruzafa E, Lendinez-Molinos F, Ortiz-Pérez M, Ruiz-Tudela L, Martín-González M (2016) Reference values of serum transferrin receptor (sTfR) and sTfR / log ferritin index in healthy children. Pediatr Hematol Oncol 33:109–120. https://doi.org/10.3109/08880018.2015.1138007
47. Vendt N, Talvik T, Leedo S et al (2009) The reference limits and cut-off value for serum soluble transferrin receptors for diagnosing iron deficiency in infants. Int J Lab Hematol 31:440–446. https://doi.org/10.1111/j.1751-553X.2008.01060.x
48. WHO (2015) The global prevalence of anaemia in 2011. In: World Heal. Organ. http://www.who.int/nutrition/publications/micronutrients/global_prevalence_anaemia_2011/en/. Accessed 9 Jan 2018
49. WHO/CDC (2007) Assessing the iron status of populations. http://www.who.int/nutrition/publications/micronutrients/anaemia_iron_deficiency/9789241596107/en/. Accessed 9 Jan 2018