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Original Article

Prevalence of Anemia, Iron Deficiency, Iron Deficiency Anemia and Diagnostic Performance of Hematologic and Biochemical Markers of Sideropenia in 1- to 5-Year-Old Children in Thrace Greece

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Abstract. ***Background and Objective:*** **Iron deficiency (ID) is a major public health problem with high prevalence in early childhood. We assessed the prevalence of anemia, ID, and iron deficiency anemia (IDA) in healthy children of Thrace, Greece, its correlation with several factors, and evaluated the diagnostic performance of hematologic and biochemical markers of sideropenia.**
Patients and Methods**: For 202 healthy children 1-5 years old, a questionnaire was filled out describing their nutritional habits during infancy and early childhood. Venous hemograms along with serum ferritin, TIBC, %TS, and CRP were obtained from all studied children. In a subset of 156 children, the concentration of sTfR was also determined.**
Results**: Children with ID and IDA had significantly lower beef consumption than children without sideropenia (*p*=0.044). Using the WHO cutoff values of Hb <11g/dl and ferritin <12μg/l, the prevalence of anemia, ID, and IDA was 9.41%, 6.44%, and 3.47%, respectively. If Hb <12g/dl and ferritin<18μg/l were used as cutoffs, the prevalence of anemia, ID, and IDA was 26.73%, 16.33%, and 5.94%, respectively. ROC analysis revealed that at ferritin <12μg/l, the AUC of sTfR alone (0.827) was substantially better than that of TIBC (0.691), while at serum ferritin cutoff of 18μg/l, the AUC of TIBC (0.770) was better than that of sTfR (0.716).**
Conclusions**: The prevalence of ID and IDA in children 1-5 years old in Thrace is like in other developed countries. The chosen cutoff of serum ferritin affects the evaluation of the diagnostic significance of the different sideropenia markers.**

Keywords: Anemia; Iron deficiency; Iron deficiency anemia; Ferritin; Sideropenia biomarkers.

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Introduction. Iron deficiency (ID) is the most common micronutrient deficiency in all countries and is a major

public health problem with high prevalence in early childhood.[1] Initially, ID leads to decreased body iron stores without anemia. However, when the iron stores are eventually depleted, iron deficiency anemia (IDA) occurs, i.e., a drop in hemoglobin (Hb) is noticed.[1] Anemia is a major public health problem worldwide, and approximately 50% of it is due to ID.[2] According to the World Health Organization (WHO), about 35% of the world's population, i.e., > 2 billion people, suffer from anemia.[3] The prevalence of ID worldwide is estimated to be 2 to 2.5 times higher than that of IDA.[4]

Three key questions arise when dealing with the diagnosis of IDA, i.e., which children should be screened for, with what hematologic and biochemical markers, and with what diagnostic cutoff values. The WHO recommends targeted screening for IDA in children before iron administration if the prevalence of anemia is >5%.[2] The American Academy of Pediatrics recommends universal screening for IDA at one year of age.[5] However, the US Preventive Services Task Force questions the value of IDA screening in asymptomatic children 6-24 months old.[6] Finally, the U.S. Centers for Disease Control and Prevention recommends targeted screening in children at high risk for IDA.[7]

Hb concentration is used for the diagnosis of anemia. However, it cannot be used as the sole marker of IDA as it lacks specificity and sensitivity.[8-10] Serum ferritin concentration is the most widely used marker of ID, as it reflects the body's iron stores with high specificity but moderate sensitivity because it increases in the presence of inflammation.[6,11] Transferrin is a hepatic glycoprotein that carries nutritional iron from the gut to sites of iron storage and the bone marrow. Transferrin saturation (%TS) is the percentage of transferrin occupied by iron.[2,11] Total iron-binding capacity (TIBC) is the maximum amount of iron that can bind to transferrin and is increased in IDA.[11] Transferrin allows the intracellular transport of iron by binding to transferrin receptors, which are transmembrane proteins found on the surface of most body cells. Soluble transferrin receptors (sTfR) are portions of transferrin receptors that circulate in the blood. When cellular iron uptake is insufficient, an elevation of TfR occurs that allows the cell to compete more efficiently for circulating iron, thus resulting in more circulating sTfR. sTfR are typically elevated in IDA and are less affected by inflammation than serum ferritin.[2,5,11] In addition, they signal the transition of subclinical ID from depleted iron stores to ineffective erythropoiesis and do not increase in serum until the body's iron stores are exhausted.[5] Therefore, the ratio of sTfR to the common logarithm of serum ferritin concentration, also known as the sTfR/Fer index, has a greater diagnostic value for IDA than the use of sTfR and ferritin alone, especially in patients with inflammatory conditions.[12] However, the above ratio has not been adequately studied in infants and children, and limited studies have been performed to determine its reference range and cutoff values for ID.[10,12-15]

In Greece, the prevalence of anemia, ID, and IDA is confounded by the high prevalence of heterozygous thalassemia and has not been well-studied during the last decade in infants and toddlers. The Thrace region is one of the least developed areas of Greece, with lower income than the rest of the country. This prospective study aimed to assess the prevalence of anemia, ID, and IDA in healthy children 1-5 years old in Thrace and correlate it with several factors. We also evaluated the diagnostic performance of hematologic and biochemical markers of ID and IDA when different cutoff values of serum ferritin and Hb were used to define ID and IDA.

Patients and Methods. From March 2019 to August 2021, we prospectively studied the prevalence of anemia, ID, and IDA in healthy children 1-5 years old. For the sample size calculation, we assumed the prevalence of ID to be around 10%. Hence, with an accuracy of ± 4% and with a confidence interval of 95%, about 200-250 children had to be studied.

Our study population included 202 healthy children 1-5 years old who lived permanently in Thrace and visited the University General Hospital of Alexandroupolis or the General Hospital of Didymoteicho for well-child visits during the study period. Children with chronic diseases, infections [serum C-reactive protein (CRP) >0.5mg/dl)], bleeding disorders, known anemia due to other causes beyond ID, and permanent residence outside Thrace were excluded. The Scientific Institutional Review Boards approved the study of both participating hospitals. The parents or guardians signed a written informed consent to provide detailed demographic and medical information and to allow laboratory testing of their children. The study's questionnaire included demographic information (child's age and sex) and information regarding parental socioeconomic status and nutritional habits during infancy and early childhood. Venous blood sampling was performed for complete blood count (CBC) measurement along with serum ferritin, TIBC, %TS, and CRP, to assess the prevalence of anemia, ID, and IDA. In a subset of 156 patients with an adequate amount of available serum, the concentration of sTfR was also determined. The Sysmex 5000 analyzer (Sysmex Corporation, Kobe, Japan) was used for CBC determination, while the Immulite 1000 analyzer (Siemens Healthcare, Erlangen, Germany) was used for serum ferritin measurement. The Targa 1500 analyzer (Biotecnica Instruments S.p.A., Rome, Italy) and the FERENE direct colorimetric method were used for TIBC and TS measurement. Finally, the ADVIA 2400 analyzer (Siemens Healthcare, Erlangen, Germany) and the immunoturbidimetry method were used to determine CRP and sTfR. The definition of WHO, i.e., Hb

Table 1. Demographics, nutritional status, and laboratory tests of healthy children and those with iron deficiency (ID) (ferritin< 12 μg/l) that were recruited.

Table 1a		Total (N=202)		Healthy Children (N=189)		Children with ID (N=13)		
		N	Relative frequency (%)	N	Relative frequency (%)	N	Relative frequency (%)	*p*-value
Sex	Males	116	57.43	110	58.20	6	46.15	0.395
	Females	86	42.57	79	41.80	7	53.85	
Age	12-35 months	86	42.57	81	42.86	5	38.46	0.757
	36-60 months	116	57.43	108	57.14	8	61.54	
Population group of Greek Thrace	Christian Orthodox	166	82.18	158	83.60	8	61.54	0.007
	Muslims (including Pomaks)	10	4.95	24	12.70	2	15.38	
	Roma	26	12.87	7	3.70	3	23.08	
Infant's diet	Breastfeeding	40	19.80	35	18.52	5	38.46	0.372
	Formula	136	67.33	24	12.70	1	7.69	
	Mixed diet	25	12.37	129	68.25	7	53.85	
	Other diet	1	0.50	1	0.53	0	0	
Fresh cow's milk consumption <12 months old (information available for 194 children)		27	13.92	26	14.36	1	7.69	0.502
Fresh cow's milk consumption >700ml / day (information available for 194 children)		66	34.02	58	32.04	8	61.54	0.038
Beef consumption / week	0	15	7.43	11	5.82	4	30.77	0.017
	1	78	38.61	73	38.63	5	38.46	
	2	90	44.55	87	46.03	3	23.08	
	3	13	6.44	12	6.35	1	7.69	
	≥ 4	6	2.97	6	3.17	0	0	

Table 1b	Total (N=202)			Healthy Children (N=189)			Children with ID (N=13)			
	N	Median (range)	Mean (±SD)	N	Median (range)	Mean (±SD)	N	Median (range)	Mean (±SD)	*p*-value
Age (months)	202	40 (12-60)		189	41 (12-60)		13	38 (17-56)		0.396
Annual family income (euros)	202	16000 (0-40000)		189	16000 (0-40000)		13	14000 (0-28000)		0.506
Duration of exclusive breastfeeding (days)	147	150 (7-420)		136	150 (7-420)		11	180 (7-180)		0.247
Age at introduction of solid foods (months)	202	6 (2-14)		189	6 (3-14)		13	6 (2-6)		0.125
Beef consumption / week	202	2 (0-7)		189	2 (0-7)		13	1 (0-3)		0.044
Hb (g/dl)	202	12.50 (7.69-15)		189	12.60 (9.30-15)		13	10.90 (7.69-13.50)		<0.0001
MCV (fl)	202	79.55 (53.10-93.20)		189	79.80 (53.10-93.20)		13	66.90 (55.40-82.60)		<0.0001
MCH (pg)	202	26.90 (17.40-36.50)		189	27 (17.40-36.50)		13	21.20 (17.40-28.50)		<0.0001
RDW (%)	197	14.70 (11.30-27.10)		185	14.60 (11.30-24.10)		12	17.65 (13-27.10)		<0.0001
Ferritin (µg/l)	202	35.60 (2.18-325)		189	38 (12.70-325)		13	8 (2.18-12)		<0.0001
% TS (%)	200		19.79 (±9.56)	187		20.46 (±9.32)	13		10.15 (±7.90)	<0.0001
TIBC (µg/dl)	201		367.70 (±70.21)	188		364.30 (±67.55)	13		417.80 (±90.30)	0.008
sTfR (mg/l)	156	1.20 (0.70-5.99)		143	1.20 (0.70-4.10)		13	1.90 (1.10-5.99)		<0.0001
sTfR/Fer index	156	0.73 (0.31-17.70)		143	0.7 (0.31-3.58)		13	2.06 (1.03-17.70)		<0.0001

SD: standard deviation; Hb: hemoglobin; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin, RDW: red cell distribution width; TS: transferrin saturation; TIBC: total iron binding capacity; sTfR: soluble transferrin receptors; sTfR/Fer: ratio of soluble transferrin receptors to log ferritin.

concentration <11g/dl for children 1-5 years old, was used to define anemia.[2] The National Health and

Nutrition Examination Survey (NHANES) serum ferritin cutoff of <12μg/l was used to delimit ID.[16] The combination of low Hb and serum ferritin was used to define IDA. Finally, the sTfR/Fer index was calculated, as previously described.[17,18]

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS), version 19.0 (IBM Corporation, Armonk, NY, USA). The normality of quantitative variables was tested with Kolmogorov-Smirnov or Shapiro-Wilks tests (for small samples). Normally distributed quantitative variables were expressed as mean ± standard deviation, while non-normally distributed variables were expressed as medians and ranges. Qualitative variables were expressed as absolute and relative (%) frequencies. For the correlation between the two independent groups (healthy children versus children with ID), the Unpaired t-test was used for variables that follow a normal distribution. Mann-Whitney U-test was used for the remaining variables. Chi-square and Fisher's exact tests were used to evaluate potential associations between qualitative variables. Receiver operating characteristic (ROC) analysis was used to evaluate the diagnostic significance of the hematologic and biochemical parameters tested. The area under the ROC curve (AUC), sensitivity, specificity, positive and negative predictive values were calculated, while Cohen's kappa was used to assess agreement. The optimal cutoff values were derived according to Youden Index. All tests were two-tailed, and statistical significance was set at $P<0.05$.

Results. The demographics, nutritional status, and laboratory tests of healthy children and those with ID are presented in **Table 1**. The children's median age was 40 months. The family's annual income ranged from 0 to 40,000 euros, with a median of 16,000 euros. Overall, 57.43% of the children were boys. Only 4.95% of the children belonged to the Muslim minority (including Pomaks) of Greek Thrace and 12.87% to the Roma minority. The median duration of exclusive breastfeeding was 150 days. The median consumption of beef was twice a week. During infancy, 19.80% of children were breastfed, 67.33% were formula-fed, and 12.37% were on a mixed diet. As shown, the median value of Hb was 12.50g/dl (7.69-15), of MCV 79.55fl (53.10-93.20), of MCH 26.90pg (17.40-36.50), and of RDW 14.70% (11.30-27.10). The mean serum ferritin was 35.60μg/l (2.18-325), of sTfR 1.20mg/l (0.70-5.99) and of sTfR/Fer index 0.73 (0.31-17.70). The mean TIBC and TS% were 367.70μg/dl (±70.21) and 19.79% (±9.56), respectively.

Overall, 23.08% of children with ID belonged to the Roma minority compared to only 3.70% of healthy children (p=0.007). Healthy children were found to have higher beef consumption than children with ID [median two meals per week (0-7) versus 1 (0–3), p=0.044]. Remarkably, 30.77% of children with ID did not include beef in their diet compared to 5.82% of healthy children (p=0.017).

The overall prevalence of anemia based on the WHO definition was 9.41%. If a cutoff of Hb<12g/dl was used, the overall prevalence of anemia was 26.73%. The overall prevalence of ID was 6.44%. If a cutoff value of ferritin<18μg/l was used, then the prevalence of ID was 16.33%. The overall prevalence of IDA was 3.47%. If Hb <12g/dl and ferritin<18μg/l were used as cutoffs, then the prevalence of IDA was 5.94%. The differences observed in the prevalence of anemia, ID, and IDA between age groups were not significant, except for the prevalence of anemia using a cutoff Hb value of 12g/dl (**Table 2**).

Table 3 and **Figures 1** and **2** depict the results of the ROC analysis for the evaluation of specificity and sensitivity of sideropenia biomarkers, i.e., %TS, TIBC, sTfR, sTfR/Fer index, and Hb in children 1-5 years old, when ferritin < 12μg/l and <18μg/l were used to define ID. When ferritin < 12μg/l was used to delimit ID, the biomarker with the highest specificity but the lowest sensitivity was sTfR (93% and 69.2%, respectively). In contrast, the biomarker with the highest sensitivity (100%) was sTfR/Fer index. sTfR were found to have the highest positive predictive value (PPV) (47.4%), while Hb was found to have the lowest PPV (16.7%). Conversely, the sTfR/Fer index was found to have the highest negative predictive value (NPV) (100%). The AUC was highest for the sTfR/Fer index (0.971), followed by sTfR (0.827). When ferritin <18μg/l was used to define ID, the sTfR/Fer index had the highest AUC (0.946).

At serum ferritin 12μg/l, as the cutoff of ID, the AUC of sTfR alone (0.827) was substantially better than that of TIBC (0.691), as shown by the green line of sTfR in **Figure 1**. On the other hand, at a serum ferritin cutoff of 18μg/l, the AUC of TIBC (0.770) was better than that of sTfR (0.716), as depicted by the blue line of TIBC in **Figure 2**.

Discussion. ID is the most common nutritional deficiency worldwide and a public health problem in late infancy and in children 2-5 years old.[1] When left untreated, IDA occurs, negatively affecting preschoolers' motor, emotional, and social development and their subsequent intellectual performance and learning abilities. Therefore, preventing ID in early childhood is a public health priority.[19] Our study used two serum ferritin thresholds, 12 μg/l and 18 μg/l, to define ID and two Hb thresholds (11g/dl and 12g/dl) to define anemia. Using the WHO cutoff value of Hb <11g/dl and of

Table 2. Prevalence of anemia, ID, and IDA based on different cutoff values of Hb and serum ferritin in 202 children, and in the subgroups of children 12-35 and 36-60 months old.

	Prevalence (%)								
	Anemia			ID			IDA		
Cut-off values	**Total**	**12-35 months**	**36-60 months**	**Total**	**12-35 months**	**36-60 months**	**Total**	**12-35 months**	**36-60 months**
Hb < 11g/dl	9.41	12.79	6.90						
Hb < 12g/dl	26.73	37.21*	18.97*						
Ferritin <12µg/l				6.44	5.81	6.90			
Ferritin <18µg/l				16.33	18.60	14.66			
Ferritin <12µg/l + Hb < 11g/dl							3.47	3.49	3.45
Ferritin <18µg/l + Hb < 12g/dl							5.94	6.98	5.17

ID: iron deficiency; IDA: iron deficiency anemia; Hb: hemoglobin. *The difference in the prevalence of anemia (Hb < 12g/dl) among children 12-35 and 36-60 months old is significant (p<0.05)

Table 3. ROC analysis for the evaluation of the diagnostic significance of transferrin saturation (%TS), total iron binding capacity (TIBC), soluble transferrin receptors (sTfR), ratio of soluble transferrin receptors to log ferritin (sTFR/Fer index), and hemoglobin (Hb) in children 1-5 years old, when ferritin < 12µg/l or ferritin <18µg/l was used to define iron deficiency.

	%TS		TIBC		sTfR		sTfR/Fer index		Hb	
Ferritin	< 12µg/l	< 18µg/l	< 12µg/l	< 18µg/l	< 12µg/l	< 18µg/l	< 12µg/l	< 18µg/l	< 12µg/l	< 18µg/l
AUC (95% CI) *p*-value	0.822 (0.678 – 0.966) <0.001	0.687 (0.583 – 0.792) 0.001	0.691 (0.517 – 0.865) 0.022	0.770 (0.684 – 0.855) <0.001	0.827 (0.682 – 0.971) <0.001	0.716 (0.607 – 0.825) <0.001	0.971 (0.942 – 1.000) <0.001	0.946 (0.912 – 0.979) <0.001	0.835 (0.711 – 0.959) <0.001	0.568 (0.443 – 0.692) 0.219
Cut-off	≤ 11.05	≤ 15.5	≥ 408.30	≥ 386.75	≥ 1.55	≥ 1.35	≥ 1.02	≥ 0.844	≤ 12.25	≤ 12.25
Sensitivity (%)	76.9 (46.0 – 93.8)	66.7 (48.1 – 81.4)	69.2 (38.9 – 89.6)	84.8 (67.3 – 94.3)	69.2 (38.9 – 89.6)	61.3 (42.3 – 77.6)	100.0 (71.7 – 100.0)	100.0 (86.3 – 100.0)	92.3 (62.1 – 99.6)	51.5 (33.9 – 68.8)
Specificity (%)	84.0 (77.7 – 88.8)	68.3 (60.6 – 75.1)	77.1 (70.3 – 82.8)	70.2 (62.6 – 76.9)	93.0 (87.2 – 96.4)	75.2 (66.5 – 82.3)	84.6 (77.3 – 89.8)	77.6 (69.1 – 84.4)	68.3 (61.0 – 74.7)	67.5 (59.8 – 74.3)
PPV (%)	25.0 (13.2 – 41.5)	29.3 (19.7 – 41.1)	17.3 (8.7 – 30.8)	35.9 (25.6 – 47.6)	47.4 (25.2 – 70.5)	38.0 (25.0 – 52.8)	37.1 (22.0 – 55.1)	52.5 (39.2 – 65.5)	16.7 (9.3 – 27.7)	23.6 (14.7 – 35.3)
NPV (%)	98.1 (94.2 – 99.5)	91.2 (84.4 – 95.3)	97.3 (92.8 – 99.1)	95.9 (90.3 – 98.5)	97.1 (92.2 – 99.1)	88.7 (80.7 – 93.8)	100.0 (96.1 – 100.0)	100.0 (95.3 – 100.0)	99.2 (95.2 – 100.0)	87.7 (80.5 – 92.6)
Overall agreement (%)	83.5	68.0	76.6	72.6	91.1	72.5	85.8	82.1	69.8	64.8
Cohen's kappa *p*-value	0.310 <0.001	0.231 <0.001	0.193 <0.001	0.356 <0.001	0.514 <0.001	0.297 <0.001	0.478 <0.001	0.579 <0.001	0.195 <0.001	0.129 0.037
OR (95% C.I.) *p*-value	17.44 (4.53 – 67.16) <0.001	4.30 (1.95 – 9.51) <0.001	7.59 (2.23 – 25.85) <0.001	13.22 (4.83 – 36.19) <0.001	29.93 (7.82 – 114.48) <0.001	4.80 (2.10 – 11.00) <0.001	–	–	25.80 (3.28 – 203.01) <0.001	2.20 (1.04 – 4.68) 0.037

ROC: receiver operating characteristics; AUC: area under the curve; CI: confidence interval; PPV: positive predictive value; NPV: negative predictive value; OR: odds ratio.

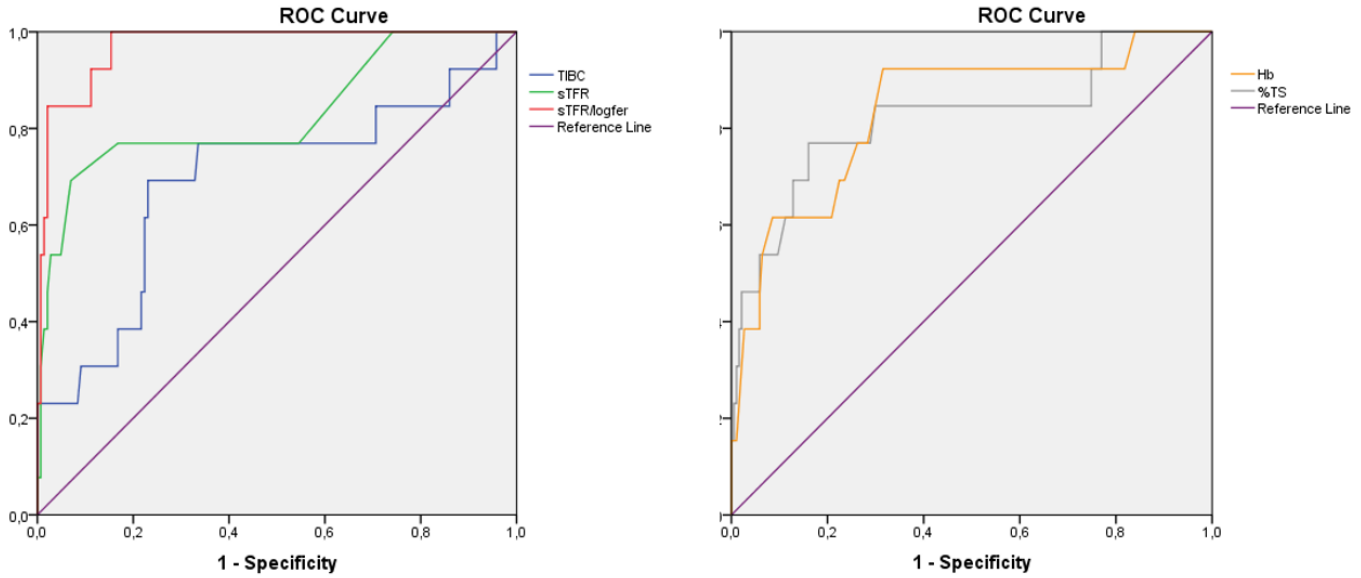

Figure 1. ROC analysis for the evaluation of the diagnostic significance of transferrin saturation (%TS), total iron binding capacity (TIBC), soluble transferrin receptors (sTfR), ratio of soluble transferrin receptors to log ferritin (sTfR/Fer index), and hemoglobin (Hb) in children 1-5 years old, when ferritin < 12μg/l was used to define iron deficiency.

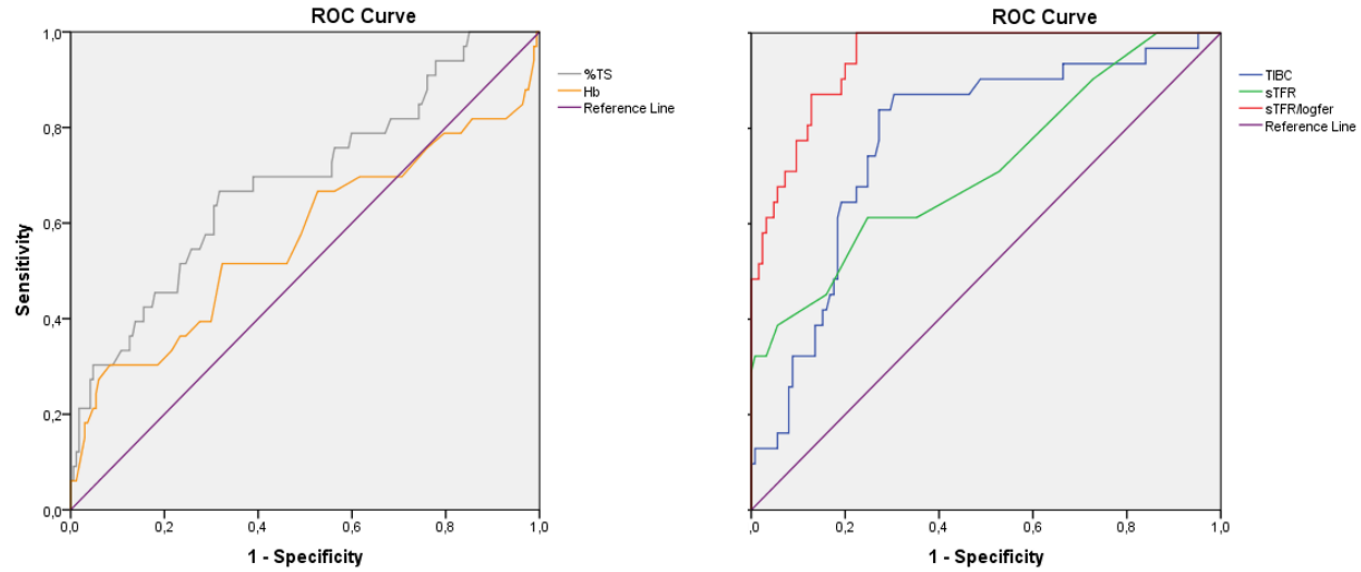

Figure 2. ROC analysis for the evaluation of the diagnostic significance of transferrin saturation (%TS), total iron binding capacity (TIBC), soluble transferrin receptors (sTfR), ratio of soluble transferrin receptors to log ferritin (sTfR/Fer index), and hemoglobin (Hb) in children 1-5 years old, when ferritin <18μg/l was used to define iron deficiency.

ferritin <12μg/l, we showed the prevalence of anemia, ID, and IDA in healthy children 1-5 years old in Thrace to be 9.41%, 6.44%, and 3.47%, respectively. Hence, only approximately 37% of anemia in healthy children 1-5 years old was IDA in our sample, an almost identical figure to the US, where 60% of anemia in toddlers is not IDA.[5] Using the higher cutoffs, the prevalence of anemia, ID, and IDA increased to 26.73%, 16.33%, and 5.94%, respectively. Higher cutoffs allow earlier intervention, i.e., dietary changes or administration of iron supplements, although their diagnostic accuracy needs to be determined prospectively via epidemiologic methods.[19] In the ROC analyses, the chosen cutoff of serum ferritin affects the evaluation of the diagnostic significance of the different markers of sideropenia. More specifically, sTfR alone was a better biomarker than TIBC when serum ferritin cutoff of 12μg/l was used to define ID, while TIBC was slightly better than sTfR at serum ferritin cutoff of 18μg/l.

A prospective long-term study in 2001 conducted in 11 European countries found the prevalence of anemia in 12-month-old infants to be 9.4%, ID 7.2%, and IDA 2.3%.[20] A review of 44 studies conducted in 19 European countries showed that ID occurred in 3-48% of children 12-36 months old, while the prevalence of IDA was close to 50% in Eastern Europe but less than 5% in Southern and Western Europe.[21] A US study published in 1997 found that the prevalence of ID and IDA in children 12-

24 months old was 9% and 3%, respectively, while in children older than three years of age, the prevalence of ID and IDA was ≤3% and <1%, respectively.[22] Similarly, in a more recent US study published in 2016, the prevalence of anemia in toddlers 1-2 years old was 2.7%, but only half of the anemic children suffered from ID. In the same study, the prevalence of ID, anemia, and IDA in healthy children 1-5 years old was 7.1%, 3.2%, and 1.1%, respectively.[23]

Regarding Greece, in a prospective study conducted in 2007 of 3,100 children aged 8 months to 15 years in Northern Greece, the prevalence of ID and IDA was 14% and 2.9%, respectively, with these rates being substantially higher in children <2 years old (34.1% and 16.1%, respectively).[24] A cross-sectional study in 2008 from Thessaly, in Central Greece, with 938 children aged 12-24 months, found the prevalence of IDA to be approximately 8%.[25,26] Notably, we did not find that the prevalence of ID and IDA was significantly different among children aged 12-35 and 36-60 months.

Hb concentration alone cannot be used to define ID or IDA, as it lacks sensitivity and specificity.[8-10] Recent studies confirm the need for combined Hb and serum ferritin testing for ID screening and verify the nonlinear relationship between them.[27,28] More importantly, these studies propose raising the diagnostic serum ferritin threshold in one-year-olds to 18μg/l from the currently accepted NHANES threshold of 10-12μg/l because, at the 18μg/l serum ferritin inflection, the Hb level is 12g/dl, i.e., much higher than the long-established WHO threshold for anemia of 11g/dl. Moreover, an anemia threshold Hb of 11g/dl corresponded to serum ferritin <5μg/l.[27,28] Thus, by allowing serum ferritin to drop to values much lower than 10-12μg/l if Hb remains >11g/dl, we lose time in correcting ID, which has potentially long-lasting neurodevelopmental consequences.[19] A single-institution study in a high-resource setting found that higher serum ferritin has been associated with higher cognitive function, with serum ferritin of 17μg/l corresponding to the maximum level of cognition at 24 months of age. However, maternal education was not included in the author's model when previous studies on cognitive outcomes of ID suggest poor maternal education and low socioeconomic status to be additional risk factors for the ID. Hence, these findings cannot be generalized to lower-income settings, and further research is essential to validate them in more diverse low- and medium-income sets.[29]

sTfR, when combined with the serum ferritin, is a useful indicator of ID and erythropoietic activity with increased specificity and sensitivity.[2,5,11] In the ROC analyses, the AUC of sTfR alone was substantially better than that of TIBC at a serum ferritin cutoff of 12μg/l, while at a serum ferritin cutoff of 18μg/l, the AUC of TIBC was slightly better than that of sTfR (0.716). Our findings are consistent with older reports in adults and children.[14,30-39] The sTfR/Fer index incorporates the high sensitivity of sTfR, which indicate cellular oxygen needs, and the high specificity of ferritin, which represents iron stores.[30] In a meta-analysis, the overall sensitivity, specificity, and positive and negative likelihood ratios of sTfR in a set of studies were 86%, 75%, 3.85, and 0.19, respectively, with an AUC of 0.912.[31] In another study, sTfR and sTfR/Fer index had the highest AUC (0.75 and 0.76, respectively). They were the most sensitive markers for detecting ID (83% and 75%, respectively) in children living in areas with a high prevalence of infections, although with moderate specificity (50% and 56%, respectively).[32] In children with inflammatory bowel diseases (IBD), the biomarkers that better-predicted ID and IDA were also the sTfR and sTfR/Fer index,[33,34] something that has been confirmed in adults with IBD as well.[35]

In our study, children with ID were found to have lower beef consumption than healthy children. In addition, 30.77% of children with ID did not include beef in their diet compared to only 5.82% of healthy children. Several studies evaluated the association between meat consumption and iron status in infants and young children, leading to conflicting results. Some found no differences in iron status when high meat consumers were compared to low meat consumers or when meat consumers were compared to cereal or milk consumers.[40] On the other hand, other studies support our findings; thus, in Northern European, healthy infants and toddlers, meat and fish consumption is associated with better iron status.[41] In a cross-sectional study of 263 Israeli healthy 1.5- to 6-year-old children, extremely low red meat consumers had a 4-fold higher rate of ID than those who consumed red meat twice per week, whereas poultry consumption was not associated with ID.[42] Moreover, a 20-week randomized placebo-controlled trial in 12-20-months-old children showed that in comparison with the control group, serum ferritin was significantly higher in the red meat group.[43] In addition, a randomized interventional trial from Denmark identified a difference in Hb but not serum ferritin when high meat consumers were compared to low meat consumers in the first year of life.[44] Finally, in a Canadian cross-sectional study of 12-36 months-old healthy children, eating meat or meat alternatives was not associated with serum ferritin but with decreased odds of ID.[40] Therefore, pediatricians should be encouraged to advocate earlier meat consumption in infants to prevent ID / IDA. However, this may not apply to low-income countries, where meat is scarce and/or too expensive to obtain regularly.

In our study, children with ID were found to have higher fresh cow's milk consumption (>700ml/24h) than healthy ones, which is consistent with current knowledge.[45] In two studies performed in Iceland, iron status at 12 months of age was negatively associated with fresh cow's milk consumption between 9 and 12 months

of age. The iron status of infants consuming higher amounts of fresh cow's milk was significantly worse than that of infants in the lowest quintile of milk consumption, suggesting the dose-dependent negative effect of fresh cow's milk on iron status.[46,47]

Our study has several limitations. First, we studied a relatively small number of children to assess the prevalence of anemia, ID, and IDA. For safer conclusions, more children had to be enrolled; but unfortunately, the recruitment period coincided with the COVID-19 pandemic, which severely limited the number of children visiting both study hospitals for well-child visits. Second, regarding most of the established environmental risk factors for sideropenia studied, no statistically significant differences were found between healthy children and those with ID, likely due to the small sample size. Third, children from the Muslim minority of Thrace were likely under-represented, although Roma children were likely over-represented. The prevalence of anemia, ID, and IDA is probably higher in minority populations. Finally, determination of sTfR was not available in all studied children.

Conclusions. We found that the current prevalence of anemia, ID, and IDA in children of Thrace 1-5 years old does not significantly differ from that of other developed countries. However, in the future, it is crucial to carefully choose the cutoff values of Hb and serum ferritin to define ID and IDA, as the goal is for fewer toddlers and preschoolers with sideropenia to remain undiagnosed and untreated. In this regard, the chosen cutoff of serum ferritin may affect the evaluation of the diagnostic significance of the different sideropenia markers.

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