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A SOLUBLE TRANSFERRIN RECEPTOR TEST PERFORMANCE IN THE DIAGNOSIS OF ID AND IDA IN CHILDREN IN CAMEROON

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ABSTRACT
This study was to determine the performance of a soluble transferrin receptor (sTfR) test for the diagnosis of iron deficiency and iron deficiency anaemia in children of 6 to 59 months. A Cross sectional multicentre study including controls was carried out in three hospitals from November 2018 to October 2019. Children of 6 to 59 months were recruited for full blood count and biochemistry tests, namely the C - reactive protein agglutination test, Ferritin and soluble transferrin receptor tests using Enzyme Linked Immuno-Sorbent Assays. Authorization was obtained from the Cameroonian national Ethical Committee and data analysis done using Statistical Package for Social sciences 21.0. Among the 383 children recruited, 288 (75.2%) had a high sTfR level, among them, those with anaemia were 160 (41.8%). Children with low Ferritin had either normal; 11 (3.8%) or high sTfR 5 (1.4%). Specificity and accuracy of sTfR in iron deficiency anaemia diagnosis were 25 and 27.2% at Ferritin cut-off value of 30µg/L. Pearson's and Spearman's correlations showed that sTfR and Ferritin levels were not associated with 0.08 and 0.047 correlation coefficients respectively. The calculation of sTfR index showed an increased specificity of the test for the diagnosis of Iron deficiency and iron deficiency anaemia. Soluble transferrin receptor as a new biomarker should be considered mostly for screening of iron deficiency anaemia, as the sensitivity was relatively high with regard to the specificity. In Cameroon the use of this new biomarker should be deeply evaluated for a good use in the future.

KEYWORDS: sTfR, sensitivity, specificity, Iron deficiency anaemia, children.

INTRODUCTION
Human cells take iron into cells from the circulation via Transferrin Receptors (TfRs). TfRs are present in human plasma.[1] These circulating soluble transferrin receptor (sTfRs) are virtually all truncated forms of cell surface TfRs. sTfR, identifed in 1986, was shown to be a single polypeptide chain with a molecular mass of 84.9 kDa.[2]

Erythropoietic activity has been found to be the most important determinant of sTfR level.[3] Decreased sTfR levels are found in situations characterized by erythroid hypoplasia such as hyper transfusion, chronic renal failure, and severe aplastic anaemia or after intensive chemotherapy. Increased sTfR levels are seen in situations of stimulated erythropoiesis, such as congenital dyserythropoietic anaemia, haemolytic anaemia, hereditary spherocytosis, sickle cell anaemia, thalassemia major or intermedia, megaloblastic anaemia or secondary polycythaemia. Levels range from a minimum of about 0.5 mg/L, the contribution of nonerythroid tissues, when erythropoiesis is totally absent, to about 100mg/L in severely anaemic thalassemia patients.[4] Serum sTfR levels are determined to detect Iron Deficiency (ID) in inflammatory states and in Anaemia of Chronic Disease (ACD) and to monitor the efficiency of Erythropoietin (EPO) treatment. The levels of sTfR reflect the receptor density on cells (tissue iron status) and the number of cells with receptors (erythropoietic activity).[5]

The diagnosis using soluble Transferrin Receptor in various studies has been shown to be more valuable biomarker than Ferritin commonly used. Experimental and clinical studies in India, Korea, China, USA and Europe showed the usefulness of this test in the diagnosis of iron deficiency anaemia.[6] In Africa the use of this biomarker is currently out looked but still far to be integrated in the diagnostic protocol. To date many assays have been developed to measure sTfR in serum and plasma. Among these Enzyme Linked Immuno

Sorbent Assays (ELISAs) have been tested and some showed good performance, however assay variability is a problem for standardization of technique and implementation of an algorithm.

In Cameroon sTfR is not used as diagnostic biomarker and assuming the good performance obtained by other studies, we found it necessary to test it in our area for the diagnosis of Iron Deficiency Anaemia (IDA) in children.

We hypothesized there is a positive correlation between IDA and soluble Transferrin Receptor (sTfR) level in children and our objective was to determine the performance of a sTfR test for the diagnosis of ID and IDA in children of 6 to 59 months considering the Ferritin test recommended by WHO as the standard.

MATERIALS AND METHODS

Study Design

This was a prospective cross sectional multicentre study carried out at the Bertoua regional Hospital, Douala Gynaeco-Obstetric and Paediatric Hospital, Yaounde University Hospital Centre from November 2018 to October 2019.

Subjects and Sampling

The target population wasb children of 6 to 59 months attending the different study sites. The sample size was calculated using the Lorentz formula. All the children of the target age visiting the paediatric unit and having a prescription of full blood count were included. Children with haemoglobin (Hb) level <11 g/ dL were considered anaemic (cases) and those with Hb ≥ 11g/dL were non-anaemic (Controls).

Measurements and Laboratory Analysis

Data were collected through a questionnaire, after a signed assent form. Once the identification of the children was done and blood sample drawn in 2ml EDTA tube for full blood count and 1 ml in dry tube for C- reactive protein (CRP), Ferritin and sTfR analysis respectively. Full blood count testing was performed using Mindray Bc-2800. Blood in dry tubes were centrifuged at 2500 G for 5 minutes to obtain serum. The serum was used onsite for CRP test by latex agglutination using Fortress Diagnostic Limited (UK) Kits following the procedure with a cut-off value of 6 mg/dL. The remaining serum was kept at – 20ºC for batch analysis of Ferritin using ERBALISA Kits by Cal Biotech Lab (USA) and sTfR using ACCUBIND ELISA Kits by Monobind Inc. USA at the biochemistry unit of the main laboratory of the Bertoua Regional Hospital.

Anaemic children were further classified into severe (Hb < 7 g/dL), moderate (Hb: 7-9.99 g/dL) and mild (Hb: 10-10.99 g/dL) anaemia. Three red cell indices were considered for microcytosis and hypochromic in red blood cells: Mean Corpuscular Volume (MCV < 80fL), Mean Corpuscular Haemoglobin (MCH < 26pg) and Mean Corpuscular Haemoglobin concentration (MCHC < 32g/dL).

Normal values for Ferritin kits, were given for men and women but not for children. We considered cut-offs of 30 and 50µg/L were considered to estimate the prevalence of ID and IDA using Ferritin the standard recommended by WHO. Anaemic children (Hb <11g/dL) were classified into 3 groups according to Ferritin level: IDA (Ferritin < 30ug/L), Mixed IDA (Ferritin 30-100ug/L), ACD (Ferritin > 100ug/L). Non anaemic children (Hb ≥ 11g/dL) were grouped into ID, Iron Deficicent Erythropoeisis (IDE) and Iron Repleted (IR) according to the Ferritin ranges described for anaemic children.

For sTfR reference values of the kit ranging from 8.7 to 28.1 nmol/L, was considered for interpretation, so from 28.1 and above children were considered positive for ID and sTfR index was calculated using the formula sTfR (in mg/L)/log10 Ferritin (µg/L) with the cut-off of 1.5 above which the children were positive. The sample size of 213 was determined through the Lorentz formula using the prevalence of 16.5% IDA found by Engle et al. in 2013, 170 Controls were added.

Ethical consideration

This study was approved by the National Ethical Committee for Research in human health of Cameroon (Ref. N°2019/09/44/CE/CNERSH/SP), the regional delegations of public health of the East and Centre regions, the scientific committee of the Douala Gynaeco-Obstetric and Paediatric Hospital.

Statistical analysis

Data were computed on Microsoft Excel 2013 and statistical analysis done on SPSS.21.0 (IBM USA). Differences and correlations between the obtained parameters were found using variable statistical tests. Mean and standard error of mean were calculated for quantitative variables, ANOVA 1 factor was used to compare means of cases and controls. To determine statistically significant differences between categories, the Chi square (x^2) and Fisher exact tests were used with a 95% confidence interval considered. Estimation of risk between cases and controls for Ferritin, CRP, sTfR and sTfR index was measured by Odds Ratios (OR). Receiver Operating Characteristics (ROC) curves were used to test the performance of the different Ferritin cut-offs used in the study. Spearman's and Pearson's correlations were applied to look for association between Ferritin and sTfR. Sensitivity, specificity, accuracy, positive and negative predictive values of sTfR and sTfR index were calculated considering Ferritin as the reference test.

RESULTS

Characteristics of the Population

The 383 children were grouped according to Haemoglobin (Hb) level, data reported as mean ±

standard error of mean (SEM). The Mean sTfR level of 50.5 ± 1.6 µg/L was found for all the children, in cases (213) and controls (170) a mean Ferritin level of 52.8 ± 3.4µg/ L and 48.4 ± 1.9 µg/L respectively were obtained with a p = 0.236 after comparing mean by ANOVA. Significant differences were noted for Hb, WBC, RBC, MCH and Ferritin with p- values of 0.0001; 0.008; 0.0001, 0.0001 and 0.001 respectively (Table 1).

Table 1: Comparison of means of haematologic and biochemical parameters between anaemic (Cases) and non anaemic (Controls) children.

Variable	Total Mean ± SEM	Anaemia (Cases)	No anaemia (Controls)	ANOVA 1-factor	p-Value
n	383	213	170		
Age	25.2 ± 0.7	25.4 ± 1.0	25.0 ± 1.2	0.037	0.847
Hb	10.4 ± 0.08	9.3 ± 0.1	11.7 ±0.05	356.844	0.0001
WBC	10340 ± 244	10916 ± 378	9618 ± 273	7.064	0.008
RBC	4250809 ± 379	3916761 ± 48932	4669353 ± 38276	135.8	0.0001
MCV	73.8 ± 0.4	73.1 ± 0.6	74.7 ± 0.5	4.8	0.38
MCH	24.6 ± 0.17	24.1 ± 0.2	25.2 ± 0.2	16.6	0.0001
MCHC	34.0 ± 0.1	34.3 ± 1.4	33.6 ± 1.5	0.225	0.625
Ferritin	290.6 ± 12.8	329.1 ± 18.6	242.2 ±16.2	11.751	0.001
sTfR	50.5 ± 1.6	52.8 ± 3.4	48.4 ± 1.9	1.409	0.236
sTfR index	1.8 ± 0.08	1.7 ± 0.2	1.89 ± 0.08	0.585	0.445

Male were more represented than female with in both groups. Most children were from 0 to 24 months (Table 2).

Table 2: Anaemia in relation with age group and gender in children.

Variable	Category	Anaemia	No anaemia	Total	Chi square
N=383		**n (%)**	**n (%)**	**n (%)**	**(p-value)**
Gender					1.267 (0.260)
	Male	125 (58.7)	90 (52.9)	215 (44.6)	
	Female	88 (41.3)	80 (47.1)	168 (55.4)	
	Total	213 (100)	170 (100)	383 (100)	
Age group					0.104 (0.950)
	6-23	103 (48.3)	81 (47.6)	184 (48.0)	
	24-41	67 (31.5)	56 (32.9)	123 (32.2)	
	42-59	43 (20.2)	33 (19.4)	76 (19.8)	
	Total	213 (100)	170 (100)	383 (100)	

Among anaemic children 157 (73.7%) suffered of ACD and in this category all the 19 (8.9%) severe cases were found. Most of the IDA 5 (2.3%) were mild and statistical analysis revealed no significant difference among the categories with a p = 0.161 (Table 3).

Table 3: Distribution of anaemic children regarding the type and the severity of anaemia.

Variable	Category	Severe	Moderate	Mild	Total	Fisher exact
N = 213		n (%)	n (%)	n (%)	n (%)	(p-value)
Type of anaemia						8.794 (0.161)
	IDA	0 (0)	4 (1.9)	5 (2.3)	9 (4.2)	
	IDA/ACD	0 (0)	26 (12.2)	21 (9.9)	47 (22.1)	
	ACD	19 (8.9)	69 (32.4)	68 (31.9)	157 (73.7)	
	Total	19 (8.9)	99 (46.5)	95 (44.6)	213 (100)	

sTfR in the diagnosis of anaemia

Inflammation tested by CRP level in serum showed that few children in this category had low Ferritin level (4; 1.1%). Anaemia did not had an impact on Ferritin and sTfR levels with a p-value of 0.958. For leukocytosis there was a significant difference in Ferritin level (p = 0.047) (Table 4).

Table 4: Association between inflammation, anaemia, leukocytosis with Ferritin and sTfR levels.

		Ferritin <30µg/L		sTfR<28.1 nmol/L		Fisher exact (p-value)
Variable N=383	**Category**	**Yes n (%)**	**No n (%)**	**Yes n (%)**	**No n (%)**	
Inflammation						2.608(0.106/*0.906)
	Yes	4 (1.1)	167 (43.5)	52(13.6)	160(41.8)	
	No	12 (3.1)	200 (52.3)	43(11.2)	128(33.4)	
Anaemia						0.003 (0.958)
	Yes	9 (2.4)	204 (53.2)	53(13.8)	160(41.8)	
	No	7 (1.8)	163 (42.6)	42(11.0)	128(33.4)	
Leukocytosis (WBC> 10000 cells/µL)						3.951(0.047/*0.404)
	Yes	3 (0.8)	161 (42.0)	58(15.1)	161(42.0)	
	No	13 (3.4)	206 (53.8)	37(9.7)	127(33.5)	

sTfR and red cell indices

Looking sTfR level and red cells indices; out of the 383 children, 239 (62.4%) had high sTfR level and were microcytic. High sTfR were observed in 219 (57.1%) and 75 (19.6%) children that were hypochromic due to MCH and MCHC respectively. There was no major statistical difference between those with low and high sTfR values $p > 0.05$ (Table 5).

Table 5: Association between red cells indices and sTfR level (nmol/L) in children.

		Low (95) sTfR <28.1	High (288) sTfR ≥ 28.1	x^2 (p-value)
Variable N=383	**Category**	**n (%)**	**n (%)**	
MCV				0.002 (0.968)
	Microcytic	79 (20.6)	239 (62.4)	
	Normal to Macrocytic	16 (2.6)	48 (12.5)	
MCH				1.618 (0.203)
	Hypochromic	66 (7.5)	219 (57.1)	
	Normal	29 (0)	69 (18.0)	
MCHC				0.003 (0.958)
	Hypochromic	25(6.5)	75 (19.6)	
	Normal	70 (18.3)	213 (55.6)	

sTfR in the diagnosis of iron deficiency and iron deficiency anaemia

Out of the 383 children, a high sTfR level was observed in 160 (41.8%) of anaemic children. Those with ACD had the highest proportion 116 (30.3%). In non-anaemic 128 (33.4%) had a high level of sTfR with most cases being IR (94; 24.8%). The difference in sTfR level was not significant between the anaemic and non ananemic groups with $p = 0.256$ and OR = 1.010 and a Confidence Interval of 0.633 -1.397 (Table 6).

Table 6: sTfR level in relation with Ferritin cut-off of 30µg/L.

sTfR (7.8-28.1 nmol/L)	Ferritin < 30µg/L (16)	Ferritin ≥ 30µg/L (367)	Fischer Exact	r (CI)
	n (%)	n (%)	(p-Value)	
			0.618 (0.432)	
Low (<7.8)	0 (0)	4 (1.0)		1.509
Normal (7.8-28.1)	11 (3.8)	277 (71.4)		(0.391-5.826)
High (>28.1)	5 (1.4)	86 (22.3)		

Out of the 383 children, 118 (30.8%) were anaemic and those with ACD having the highest rate 81 (21.1%). In the non-anaemic group 94 (24.5%) had high sTfR index level with the highest rate found amongst those with IR (63; 16.4%). There was no significant difference in sTfR index between the anaemic and non-anaemic group observed in 30.8% of anaemic and those with ACD showed the highest rate 81 (21.1%) with $p = 0.137$ and OR = 0.932 (0.621-1.397) (Table 7).

Table 7: Distribution of sTfR index at a cut-off 1.5 according to group in children.

		sTfR Index < 1.5 (171)	sTfR Index ≥ 1.5 (212)	x^2 (p-value)	OR (CI)
Variable N=383	**Category**	**n (%)**	**n (%)**		
Anaemia				8.363 (0.137)	
	IDA	3 (0.8)	6 (1.6)		
	Mixed IDA	16 (4.2)	31 (8.1)		
	ACD	76 (19.8)	81 (21.1)		
	Total	95 (24.8)	118 (30.8)		0.932 (0.621-1.397)
No anaemia					
	ID	2 (0.5)	5 (1.3)		
	IDE	14 (3.6)	26 (6.8)		
	IR	60 (15.7)	63 (16.4)		
	Total	76 (19.8)	94 (24.5)		

Among the 383 children tested, a high sTfR level observed in 41.8% (160) of anaemic and those with ACD showed the highest proportion 119 (30.3%); in non ananemic 33.4% (128), the most represented group being the IR with 24.8% (94). The difference in sTfR level was not significant between groups with p = 0.256 and OR = 1.010 (0.633 – 1.397) for anaemia and no anaemia (Table 8).

Table 8: Distribution of sTfR level (cut-off 28.1 nmol/L) according to group in children.

		Low <28.1 nmol/L	High ≥28.1 nmol/L	x^2 (p-value)	OR (CI)
Variable N=383	**Category**	**n (%)**	**n (%)**		
Anaemia				6.555 (0.256)	
	IDA	2 (0.5)	7 (1.8)		
	Mixed IDA	10 (2.6)	37 (9.7)		
	ACD	41 (10.7)	116 (30.3)		
	Total	53 (13.8)	160 (41.8)		1.010 (0.633-1.397)
No anaemia					
	ID	2 (0.5)	5 (1.1)		
	IDE	11 (2.9)	29 (7.3)		
	IR	29 (7.6)	94 (24.8)		
	Total	42 (11.0)	128 (33.4)		

Diagnostic performances of the test using sTfR and sTfR index levels

Considering the data in tables 7 and 8, ID was determined in the 383 children with 2 different levels of Ferritin (<30 µg/L for simple ID and < 100 µg/L for ID + IDE), we obtained a sensitivity of 75 and 75.7% respectively using sTfR level and specifity of 24.8% and 25% . Regarding sTfR Index, sensitivity of 68.8 and 66.0% lower than that of sTfR and greater specificity values of 46.2 and 53.3%.were obtained for ID.

IDA was calculated for the 213 children with 2 different levels of Ferritin (<30 µg/L for simple IDA and < 100 µg/L for IDA + Mixed IDA), we had a sensitivity of 77.8 and 78.6% respectively, specificity for the 2 levels of Ferritin was 25.4 and 26.1%. Using the sTfR index we had low sensitivity of 66.7 and 66.1% respectively and specifity values of 46.0 and 53.1%. Low specificity and accuracy of sTfR and sTfR index observed for the different level of Ferritin. (Table 9).

Table 9: Diagnostic performances of sTfR and sTfR index in ID and IDA in children.

Variable	Category	Se (%)	Sp (%)	Acc (%)	PPV (%)	NPV (%)
sTfR	**ID**					
	Ferritin <30µg/L	75.0	24.8	26.9	4.2	95.8
	Ferritin <100µg/L	75.7	25.0	38.6	27.1	90.5
	IDA					
	Ferritin <30µg/L	77.8	25.4	27.2	4.4	96.2
	Ferritin <100µg/L	78.6	26.1	39.9	27.5	77.4
sTfR Index	ID					
	Ferritin <30µg/L	68.8	45.2	46.2	5.2	97.1
	Ferritin <100µg/L	66.0	48.6	53.3	32.1	79.5
	IDA					
	Ferritin <30µg/L	66.7	45.1	46.0	5.1	96.8
	Ferritin <100µg/L	66.1	48.4	53.1	31.3	80.0

Sensitivity (Se), Specificity (Sp), Accuracy (Acc), Positive Predictive Value (PPV), Negative Predictive Value (NPV).

DISCUSSION

Limitations

This study was done with hurdles in some aspects of iron deficiency anaemia diagnosis. This include the diagnosis of anaemia through simple blood count without doing thin smear, use of soluble transferrin receptor alone as additional diagnostic tool of iron status, iron intake and anthropometric parameters not measured to know the real nutritional status of the children at the time of recruitment and common infections as malaria and hookworm not tested to elucidate their impact on Ferritin.

Male sex and the younger age mostly represented in our study, are consistent with the study of Moschovis et al while studying risk factors among young children in sub-Saharan Africa[7], this may be explained by the accelerated growth velocity during the first year of life, since growth velocity is higher in boys when compared to girls during this period.[8]

Diagnostic performance of sTfR using Ferritin as reference

sTfR as a biomarker for the diagnosis of IDA was not indicated as we had a low sensitivity and specificity rate in our study population. A high number of children had high levels of this biomarker. We noticed further that without counting the effect of inflammation on Ferritin, simple cases of IDA were not all detected using sTfR (Table 2). Indeed under conditions where iron stores are reduced (Low Ferritin) there is a demand in iron entering, characterized by an increase expression of specific receptors as soluble transferrin receptor.[9] But here only 5/16 could be detected as having low Ferritin level and high sTfR level concomitantly.

In our study there was a general increase in sTfR regardless of inflammatory status, anaemic conditions or low Ferritin. This level could be normal as it is stated in the literature that Africans had naturally elevated level of sTfR.[10] The prevalence of IDA while considering high sTfR as criteria was 41.8% a rate slightly lower than the one obtained by Engle team's with 47.8%.[11] This suggested that majority of children were suffering of mixed anaemia and could then not be easily diagnosed if the testing criteria was Ferritin. Additionally the increase specificity while using sTfR index underlined the need of associating Ferritin and sTfR for a more accurate diagnosis of ID and IDA.

Using sTfR as a biomarker in the diagnosis of IDA has been proved to be an interesting pathway to improve the standard, as confirmed by most authors.[12-14] It was presented as a marker not altered by inflammation and justified in our case with no significant difference found in sTfR levels in children with or without inflammation. This add value to anterior findings presenting Ferritin as a weak biomarker considering the fact that it will increase or stay normal in case of infection and inflammation.[15] Malaria being the disease most concerned with the symptom of anaemia, low iron stores instead of being deleterious has been identified as a protective factor in children. Meaning children with iron deficiency anaemia seems to resist to malaria infection, but on the other hand when the infection succeed to surrender the immune system, the disease is severe and consequences could be fatal.[16]

The low sensitivity and specificity obtained using the Accubind Elisa kits might be attributed to the lack of standardization in assay to facilitate the use of valid Kits and the scarceness of this cut edge reagent in Cameroon. The low sensitivity (68.7%) showed that the test failed to find iron deficiency in more than 30 children out of a 100 and could confirmed the effective iron store 25 out of 100 children with a specificity of 24.6%.

For IDA there was an increase of sensitivity (77.8%) and almost no change with the specificity (25%), showing that with anaemia about 80 children can be detected of IDA on the basis on sTfR alone. But exploration of iron status in children with normal iron stores using this sTfR yield poor performance. With the purpose of testing if an increase in Ferritin cut off could improve the diagnosis of iron deficiency and IDA, we found there was a change but the difference was not significant to validate the fact that with high Ferritin cut off, the sTfR had an undisputable sensitivity and specificity to be used in

hospital for the diagnosis. The use of ROC curves to test the efficacy of sTfR considering Ferritin cut-offs of 30 and 50 µg/L, showed that sTfR independently of the level of Ferritin had poor performance and was more as a random testing(Table 3).

Considering the fact that the used kit was not among the tests assessed by WHO[17] which selected the following kits: Beckman Coulter, Inc., Dade Behring Marburg GmbH, Orion Diagnostica, R&D Systems, Ramco and Roche for qualification. Therefore we questioned the specificity and avidity of the coated antibodies of the used kit as they could be the cause of cross reactivity thus making the sensitivity to be three times higher than the specificity.

Though there is a controversy in the use of sTfR as a single biomarker for the detection of iron deficiency and iron deficiency anaemia, these results showed that sTfR failed to diagnosed early stage of low iron store and therefore may not be good for prophylactic action in children. However it showed better sensitivity performance while considering IDA, thus increasing the potential of sTfR as a good screening biomarker but the low specificity may outline the fact that this test should not be considered as a confirmatory test, but could be oriented for confirmation when associated with Ferritin as sTfR index. Additionally as biotechnologists are working actively in promoting rapid diagnostic testing[18], the tests designed to assess sTfR can be used for screening but should be overlooked or combined with other tests when there is a need of confirmation.

Definitely not affected by inflammation, there is however a black point on the biological relationship between Ferritin and sTfR. The correlation between sTfR and Ferritin was not confirmed through the tests of Spearman and Pearson.

Some researchers initially stated that when Ferritin is low, sTfR is high but in our results there was neither negative correlation as to show there is an inverse interaction for these 2 parameters Pearson's correlation of 0.080, nor an association showing that they interact in normal conditions with a Spearman's Correlation of 0.047. However the risk of having a high sTfR level was increased in children with low Ferritin. This controversy add value to the statement of Guzman et al on the fact that in the body the interplay between iron, iron stores, Ferritin and sTfR is not fully understood and many hypothesis are developed.[19]

CONCLUSION

Ferritin recommended by WHO for ID and IDA Diagnosis was not used in our health settings. Full blood count currently used should be interpreted cautiously as only MCV and MCH corroborate with a Ferritin level lower than 30 µg/L, sTfR and sTfR index.Soluble transferrin receptor (sTfR) as a new biomarker should be considered mostly for screening of ID and IDA

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Competing Interests

Authors declared there is no competing interests in this study.

Authors' Contributions

Agokeng D. Sylvie: designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript.
Tayou T. Claude: managed the analyses of the study, managed the literature searches and reviewed the first draft of the manuscript.
Assob N. Clement: supervised the study and reviewed the first draft of the manuscript.
NJunda Anna L.: supervised the study and reviewed the first draft of the manuscript.
All the authors agreed for the publication of this manuscript.

Consent

A parental agreement was needed before children participation in the study and a clear consent given by the parent/guardian before selecting the children as participant in accordance with inclusion criteria.

Ethical approval

This study was approved by the National ethical committee for health under an ongoing research on diagnostic biomarkers of iron metabolism, namely soluble transferrin receptor. All the data collected from the research were codified, kept confidential and analysed anonymously.

REFERENCES

1. Camaschella C. Iron and hepcidin: a story of recycling and balance. Hematology Am Soc Hematol Educ Program, 2013; (Table 1): 1–8.
2. Kohgo Y, Niitsu Y, Kondo H, Kato J et al. Serum transferrin receptor as a new index of erythropoiesis. Blood, 1987; 70: 1955–8.
3. Thambiah SC, George E, Aini UN, Sathar J et al. Serum soluble transferrin Surrogate marker of adequate transfusion in adult beta-thalassaemia intermedia patients. Malaysian J Med Health Sci, *2013;* 9(2): 3-12.
4. Beguin Y. Soluble transferrin receptor for the evaluation of erythropoiesis and iron status. Clinica Chimica Acta, 2003; 329(1–2): 9–22.
5. Speeckaert MM, Speeckaert R, Delanghe JR. Biological and clinical aspects of soluble transferrin receptor. Crit Rev Clin Lab Sci, 2010; 47(5-6): 213-28.
6. Yoon SH, Kim DS, Yu ST, Shin SR et al. The usefulness of soluble transferrin receptor in the

diagnosis and treatment of iron deficiency anemia in children. Korean J Pediatr, 2015 Jan 15; 58(1): 15–9.
7. Peter P Moschovis, Matthew O Wiens, Lauren Arlington, Olga A Douglas et al. Individual, maternal and household risk factors for anaemia among young children in sub-Saharan Africa: a cross sectional study. BMJ Open, 2018; **8**: e019654. doi: 10.1136/bmjopen-2017-019654.
8. Gisele A Bortolini, Márcia R Vitolo. Relationship between iron defciency and anemia in children younger than 4 years. J Pediatr (Rio J), 2010; 86(6): 488-492.
9. Infusino I, Braga F, Dolci A, Panteghini M. Soluble transferrin receptor (sTfR) and sTfR/log ferritin index for the diagnosis of iron-deficiency anemia: A meta-analysis. Am J Clin Pathol, 2012; 138(5): 642–649.
10. Sherwin DeSouza, Anita Shet, Prasanna K Kapavarapu and Arun S. Shet. Evaluating biomarkers of iron deficiency anemia in anemia of inflammation. Blood, 2013; 122: 21,948.
11. Engle-Stone R, Nankap M, Ndjebayi AO, Erhardt JG, et al. Plasma Ferritin and Soluble Transferrin Receptor Concentrations and Body Iron Stores Identify Similar Risk Factors for Iron Deficiency but Result in Different Estimates of the National Prevalence of Iron Deficiency and Iron-Deficiency Anemia among Women and children in Cameroon. J Nutr, 2013 ; 143(3): 369–77.
12. Timo T. Timo Takala Soluble Transferrin Receptor role in detection of iron deficiency. Annales universitatis turkuensis tom. 1322 | Medica - Odontologica | Turku, 2017; 25-30.
13. Fady M. El-Gendy, Mahmoud A. El-Hawy, Mohamed S. Rizk, Sally M. El-Hefnawy et al. Value of Soluble Transferrin Receptors and sTfR/log Ferritin in the Diagnosis of Iron Deficiency Accompanied by Acute Infection. Indian J Hematol Blood Transfus, 2018; 34(1): 104–109.
14. Okpokam D Chioma, Osim E Efiom, Usanga E Achibong, Emeribe A Uchenna et al. Correlation Between Soluble Transferrin Receptor Versus Haemoglobin Concentration, Transferrin Saturation and Serum Ferritin Levels Following Repeated Blood Donations in Nigeria. Asian J. Biol. Sci, 2018; 11(1): 9-15.
15. Drakesmith H. Next-Generation Biomarkers for Iron Status. Nestle Nutrition Institute workshop series, 2016; 84: 59-69.
16. Goheen MM, Wegmüller R, Bah A, Darboe B et al. Anemia Offers Stronger Protection Than Sickle Cell Trait Against the Erythrocytic Stage of Falciparum Malaria and This Protection Is Reversed by Iron Supplementation**.** EBio Medicine, 2016; 14: 123 DOI: 10.1016/j.ebiom.2016.11.011.
17. Susan J Thorpe, Giles Sharp and Alan Heath, Mark Worwood, James Cook. WHO Reference Reagent for the Serum Transferrin Receptor (sTfR). Expert committee on biological standardization Geneva, 19 to 23 October 2009.
18. Srinivasan B, Julia L. Finkelstein, Dakota O'Dell, Erickson D, Mehta S. Rapid diagnostics for point-of-care quantification of soluble transferrin receptor. EBioMedicine, 2019; 42: 504–510.
19. Guzmán Llanos, M J Guzmán Zamudio, J LLanos de los Reyes-García. Significance of anaemia in the different stages of life. Enfermería Global. 2016; 43: 419-430.