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Soluble transferrin receptor-ferritin index in the evaluation of anemia in inflammatory bowel disease: a case-control study

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Abstract

Background No reliable biochemical markers exist for the differentiation between iron deficiency anemia (IDA) and anemia of chronic disease (ACD) in the setting of inflammatory bowel disease (IBD). The aim of this study was to investigate the use of soluble transferrin receptor (sTfR) and sTfR-ferritin (sTfR-F) index in the evaluation of anemia in patients with IBD.

Methods One hundred IBD patients [49 ulcerative colitis (UC), 51 Crohn's disease (CD)] and 102 healthy controls were enrolled. Serum levels of ferritin, transferrin saturation and sTfR were analyzed in all patients and controls. sTfR-F index was calculated based on the ratio: sTfR/$\log_{10}$ ferritin. The value of sTfR and sTfR-F for diagnosis of IDA was assessed.

Results Forty two IBD patients (41% of UC and 42.9 % of CD) fulfilled the WHO criteria for the diagnosis of anemia**.** Among them thirty (30 %) had IDA, four (4%) had ACD and eight (8%) had mixed IDA/ACD. Patients with IDA had significantly higher sTfR and sTfR-F index levels compared with those without IDA (P<0.0001). Both sTfR and sTfR-F index were not correlated with CRP levels or disease activity. High sTfR levels (>1.8 mg/L) had sensitivity 81% and specificity 80%, whereas high sTfR-F index (>1.4) had sensitivity 91% and specificity 92% for the diagnosis of IDA.

Conclusion These results suggest that the sTfR-F index seems to be very efficient in the detection and diagnosis of IDA, among patients with IBD.

Keywords anemia, Crohn's disease, ferritin, iron deficiency, ulcerative colitis

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Introduction

The pathophysiological background of anemia in patients with inflammatory bowel disease (IBD) has been established to represent two main types: iron deficiency anemia (IDA) and anemia of inflammation, or anemia of chronic disease (ACD), with the former being the most prevalent and common [1,2].

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Conflict of Interest: None

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Many IBD patients present with a mixed-type anemia, where both types participate to a variable extent, due to the ongoing inflammation which takes place in the level of enterocyte, even in patients in clinical and biochemical remission [3,4]. This mixed-type anemia is one of the most frequent and difficult clinical problems to diagnose and treat for the IBD practitioner, due to the difficulty to determine the extent to which each type is responsible for the final clinical setting in the individual patient [5]. Unfortunately, conventional biochemical markers for the evaluation of iron status, such as ferritin and transferrin, in these patients are frequently unreliable, because they are influenced by the presence of inflammation and therefore not useful in the diagnosis of anemia in the case of IBD. On the other hand, a variety of new generation indices (hepcidin, percentage of hypochromic red cells, reticulocyte hemoglobin concentration, immature reticulocyte fraction, red blood cell size factor and reticulocyte distribution width) are under investigation in the literature and some of them seem very promising in the evaluation of anemia in patients with IBD [6,7].

Soluble transferrin receptor (sTfR) is a truncated form of the cellular transferrin receptor, without its transmembrane and cytoplasmic domains, and circulates bound to transferrin [8]. The number of sTfR reflects the cellular requirements for iron, and varies with the function and the morphological development of each cell type. In IDA the numbers of sTfR increase significantly, whereas the serum concentration of sTfR is an indicator of the iron supply available for erythropoiesis; sTfR reflects erythropoiesis and inversely correlates with the amount of iron available for erythropoiesis [9-11]. It seems that sTfR is not influenced by chronic or acute inflammation; therefore it could be a more reliable index in diagnosing IDA in patients with IBD [9,12]. There is a growing amount of literature concerning the role of the sTfR/ferritin or sTfR/log ferritin ratio (sTfR-F index) in the evaluation of anemia in patients with chronic inflammatory diseases, especially diagnosing IDA in the setting of chronic inflammation or discriminating iron deficiency in the absence of anemia [11,13]. It seems that the sTfR-F index has a higher diagnostic power than either sTfR or ferritin alone [14]. To the best of our knowledge, there is as yet no study in the literature evaluating the sTfR-F index in patients with IBD.

The aim of this study was to investigate the clinical usefulness of the sTfR-F index in the evaluation of anemia in patients with IBD and especially for differentiation between IDA and ACD.

Materials and Methods

Patients

One hundred consecutive IBD patients followed up at the Gastroenterology Department of the University Hospital of Heraklion were included in the study. They were compared with 102 healthy controls (HC) of similar age. The main demographic and clinical parameters of the patients and HC are shown in Table 1. HC were all recruited from healthy blood donors, with no significant medical history or family history of IBD. All patients and controls were of Caucasian origin. Patients with hemoglobinopathy or thalassemia trait, history of GI bleeding, renal insufficiency or hematologic, liver and autoimmune disorders, malignancy, chemotherapy or radiotherapy, as well as females with ongoing pregnancy or recent delivery (<12 months from the study initiation) were excluded from the study. Finally, none of the patients or HC had received blood transfusion or treatment with iron or erythropoietin during the last three months, before the study initiation.

Diagnosis of ulcerative colitis (UC) and Crohn's Disease (CD) was based on latest European Crohn's and Colitis Organization (ECCO) criteria [15,16], while the Montreal classification [17] was used for disease phenotyping. Disease activity was calculated at the time of blood collection; for CD patients, the CDAI score [18] was used for dividing them into two groups: "Active" with CDAI>150 and "Inactive" with CDAI<150. In UC patients, the Simple Clinical Colitis Activity Index (SCCAI) [19] was used for evaluation of disease activity and "Active" disease was considered with a score of 3 or more. Serum CRP levels were also used as a marker of evaluation of disease activity. Definition of anemia was based on the established WHO criteria of hemoglobin <12 g/dL and <13 g/dL, for non-pregnant women and for men respectively [20]. None of the healthy controls was anemic.

Informed consent was obtained from all patients, the study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki (6th revision, 2008) and was approved by the Ethics Committee of the Medical Faculty of Crete.

Laboratory studies

Whole blood samples were collected from patients and HC for a full blood count and erythrocyte sedimentation rate (ESR) measurement at the time of collection. All full blood counts were processed using the COULTER® LH780 Hematology Analyzer (Beckman Coulter, Inc., CA, USA), according to the manufacturer's instructions. Serum samples were separated at the same time by centrifugation at room temperature for measurement of the following laboratory parameters: C-reactive protein (CRP), iron (Fe), ferritin (Fer), transferrin (TRF), and transferrin saturation (Tsat). All measurements were processed according to standard laboratory practice. sTfR was measured by commercially available enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, USA). sTfR-F index was calculated based on the ratio: sTfR/$\log_{10}$ ferritin.

Statistical analysis

All results are expressed as means ± standard deviation or median presented together with the interquartile range (IQR: 25th percentile, 75th percentile). Comparisons among the three diagnostic groups in terms of continuous measurements were made by the Kruskal-Wallis test (nonparametric ANOVA). Post hoc multiple comparisons tests were made by Dunnett's test. Comparisons between two groups were made by either the Student's t-test or Mann-Whitney U test. Kolmogorov and Smirnov test was used to assess the assumption that data were sampled from populations that follow the Gaussian distributions. The association between sTfR or sTfR-F index and other serum markers as well as with clinical parameters was examined by non parametric correlation (Spearman's *r*). The sensitivity, specificity and positive and negative predictive values were estimated to assess the validity of these markers in diagnosing IDA. All statistical calculations were processed using the MedCalc software package (MedCalc software, Belgium). A level of P <0.05 was considered statistically significant.

Results

Forty-two IBD patients (41% of UC and 42.9 % of CD) fulfilled the WHO criteria for the diagnosis of anemia**.** Among

Table 1 Demographic and clinical characteristics of the patients and controls included in the study

	UC	CD	IBD total	HC
Number	49 (49%)	51 (51%)	100	102
Mean age (years SD)	50.1±14.9	48.2± 16.9	49.1± 15.9	45.5 ±10.1
Male	32 (65.3%)	26 (51%)	58 (58%)	82 (80.4%)
Female	17 (34.7%)	25 (49%)	42 (42%)	20 (19.6%)
Current smokers	9 (18.4%)	21 (41.2%)	30 (30%)	58 (56.9%)
Disease activity				
Active	11 (22.4%)	9 (17.6%)	20 (20%)	
Inactive	38 (77.6%)	42 (82.4%)	80 (80%)	
Median disease duration* (years, IQR)	8.0 (5-15.7)	10.0 (4-16.3)	9.0 (4-16)	
Disease localization				
Proctitis(UC)/Ileum(CD)	14 (28.6%)	21 (41.2%)		
Left-sided(UC)/Colon(CD)	23 (46.9%)	13 (25.5%)		
Extensive(UC)/Ileum+Colon(CD)	12 (24.5%)	16 (31.4%)		
Upper GI involvement		1 (1.9%)		
Disease type (CD)				
Stenotic		11 (21.6%)		
Fistulizing		8 (15.7%)		
Inflammatory		32 (62.7%)		
Perianal disease		6 (11.8%)		
Extraintestinal manifestations	7 (14.3%)	26 (51%)	33 (33%)	
Current treatment				
Salazopyrine	7	9	16	
5-ASA	41	19	60	
Oral steroids	2	16	18	
Azathioprine	10	21	31	
Methotrexate	3	4	7	
Infliximab	2	10	12	
Metronidazole	0	6	6	

**not normally distributed*
CD, Crohn's disease; HC, healthy controls; UC, ulcerative colitis; 5-ASA, 5-amino salicylic acid; SD, standard deviation; IQR, interquartile range

them thirty (30 %) had IDA (ferritin<30 μg/L and Tsat<16 %), 4 (4%) had ACD (ferritin >100 μg/L and Tsat<16 %) and 8 (8%) mixed IDA/ACD (ferritin: 30-100 μg/L and Tsat<16 %).

Table 2 shows the measured laboratory parameters of UC and CD patients compared with healthy controls. Significant differences concerning all examined parameters (hemoglobin, MCV, ferritin, Tsat, sTfR, sTfR-F index, CRP and ESR) between both UC and CD patients and healthy controls were observed. No differences between UC and CD were found. Ferritin levels significantly correlated with CRP levels (r=0.19, P=0.04) but did not correlate with clinical indices of disease activity.

Patients with IDA had significantly higher sTfR levels compared with those without IDA (patients with other causes of anemia and patients without anemia) [median 2.6 mg/L (range 1.2-6.8 mg/L) vs. 1.2 mg/L (0.7-3.1 mg/L), P<0.0001)] (Fig. 1). Similarly, patients with IDA had significantly higher

Table 2 Laboratory data of IBD patients included in the study compared with healthy controls

Parameter Mean ± SD	UC	CD	HC	p
Hemoglobin (g/dL)	13.1 ± 2.0	12.6 ± 1.9	15.0 ± 1.2	<0.0001
MCV (fl)	86.4 ± 8.1	87.6 ± 11.0	90.1 ± 5.5	0.01
Ferritin* (μg/L, median, IQR)	42.1 (22.1-106.4)	33.4 (14.4-80.8)	71.0 (36.2-121.1)	0.003
Tsat (%)	20.4 ± 10.6	16.9 ± 12.8	32.2 ± 15.1	<0.0001
sTfR* (mg/L, median, IQR)	1.3 (1.1-2.0)	1.4 (1.1-1.9)	1.1 (0.9-1.2)	<0.0001
sTfR-F* (median, IQR)	0.9 (0.6-1.3)	0.9 (0.6-1.7)	0.6 (0.5-0.8)	<0.0001
CRP*(mg/dL, median, IQR)	0.37 (0.32-0.77)	0.39 (0.32-1.4)	0.29 (0.29-0.32)	<0.0001
ESR (mm/h)	22.8 ± 19.2	28.0 ± 16.2	6.9 ± 5.7	<0.0001

**not normally distributed*

CD, Crohn's disease; HC, healthy controls; UC, ulcerative colitis; MCV, Mean corpuscular volume; Tsat, transferrin saturation; sTfR, soluble transferrin receptor; sTfR-F, soluble transferrin receptor-ferritin index; CRP, C-reactive protein; IQR, interquartile range; ESR, Erythrocyte Sedimentation Rate.

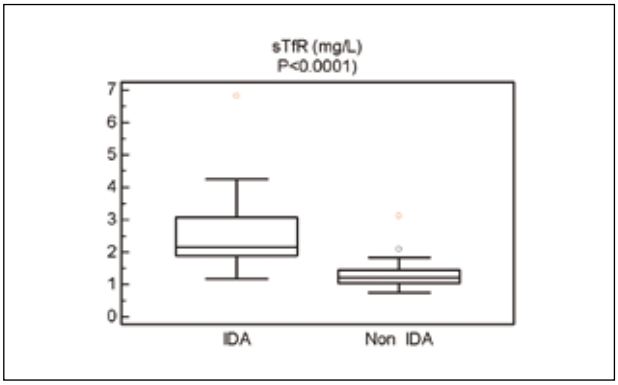

Figure 1 Distribution of soluble transferrin receptor (sTfR) levels in patients with inflammatory bowel disease (IBD) and iron deficiency anemia (IDA, n=30) and IBD patients without IDA (Non IDA, *n*=70). Boxes indicate the interquartile range with median value. Bars show the 5th and 95th percentiles.

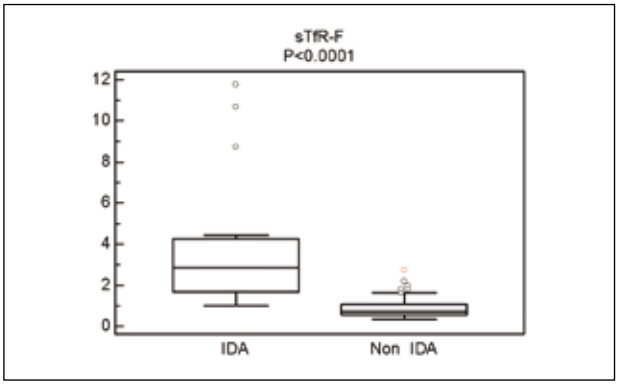

Figure 2 Distribution of soluble transferrin receptor –ferritin (sTfR-F) index in patients with inflammatory bowel disease (IBD) and iron deficiency anemia (IDA, n=30) and IBD patients without IDA (Non IDA, *n*=70). Boxes indicate the interquartile range with median value. Bars show the 5th and 95th percentiles.

sTfR-F index compared with those without IDA [2.9 (1.0-11.8) vs. 0.7 (0.4-2.8), P<0.0001)] (Fig. 2). Moreover, IBD patients with IDA had significantly higher sTfR and sTfR-F index levels compared with IBD patients with ACD (including mixed IDA/ACD) (P<0.0001).

The ability of sTfR and sTfR-F index to identify IDA among IBD patients with anemia compared to IBD patients without anemia was examined by means of receiver operating characteristic (ROC) analysis (Fig. 3). The sensitivity of high sTfR levels for diagnosis IDA using a cutoff of 1.8 mg/L was 81%, the specificity was 80%, whereas positive predictive value was 63% and negative predictive value was 91%. The sensitivity of high sTfR-F index for diagnosis IDA using a cutoff of 1.4 was 91% and the specificity was 92%, whereas positive predictive value was 83% and negative predictive value was 96%.

No correlation between sTfR or sTfR-F index and CRP levels was found (r=0.03, P=0.77 and r=0.01, P=0.28 respectively). Moreover, there were no significant associations between

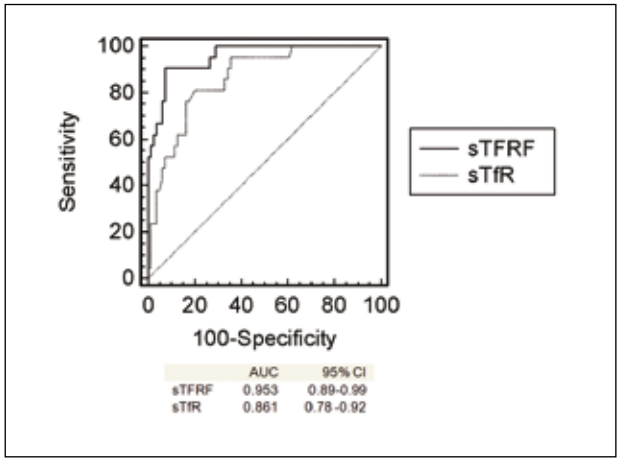

Figure 3 Receiver operating characteristic (ROC) curves for soluble transferrin receptor (sTfR) and sTfR –ferritin (sTfRF) index, in the diagnosis of iron deficiency anemia in patients with inflammatory bowel disease

sTfR or sTfR-F index and other clinical characteristics of the patients (sex, age, smoking status, disease duration, disease activity, extraintestinal manifestations and use of medications including biological therapy).

Discussion

There is a substantial difficulty in the interpretation of the iron status indices in patients with IBD, because of the presence of a continuous inflammatory process, which interferes with the value of some established markers, such as ferritin and transferrin. Therefore, the need for more convenient markers, which could more reliably differentiate the group of patients with IDA, has made researchers develop new parameters for this purpose. sTfR seems particularly useful for diagnosis of IDA in patients with infection, inflammation or malignancy where serum ferritin is not a good indicator of iron deficiency [21]. Unlike ferritin and transferrin, chronic inflammation and hepatic damage have no effect on sTfRs, which should make them a more reliable parameter than serum ferritin for diagnosing IDA in patients with IBD [9,12].

In this study we evaluated the accuracy of the sTfR-F index in diagnosing IDA in patients with IBD. This is the first study evaluating the sTfR-F index in this group of patients. We found that both sTfR and sTfR-F index were significantly increased in patients with CD and UC, compared with HC. Patients with IDA had significantly higher sTfR and sTfR-F index levels compared with those without IDA (patients with other causes of anemia and patients without anemia). Moreover, IBD patients with IDA had significantly higher sTfR and sTfR-F index levels compared with IBD patients with ACD (including mixed IDA/ACD). High sTfR-F index (>1.4) had a better discriminating power (sensitivity 91%, specificity 92 %) than high sTfR levels (>1.8 mg/L) (81% & 80% respectively) in the diagnosis of IDA.

Our results seem to be in accordance with the literature about sTfR-F index, although there are few published reports. A high diagnostic power of sTfR or sTfR-F index for differentiating IDA from ACD and mixed anemia has been suggested by several studies in various groups of patients [12, 14, 22-24]. The sTfR-F index has been suggested to be able to differentiate more accurately between IDA and ACD or to assess the iron status in patients with mixed type anemia [11]. In a study with 96 patients with rheumatoid arthritis [25], the results indicated that sTfR-F index could be used to help differentiate coexisting IDA in patients with ACD. ROC analysis showed a higher discriminating power of sTfR-F index vs. sTfR in the diagnosis of iron deficiency anemia, as well as in the differential diagnosis between IDA and ACD. Vazquez Lopez et al [26] reported a significantly increased sTfR-F index in children with storage iron deficiency, compared to healthy children with normal iron status, presenting an AUC of 0,96 for this index and sensitivity and specificity of 89% and 96% respectively, with 90% PPV and 94% NPV for discriminating IDA, when the index was >2. In another study [27] the above ratio is suggested as a useful alternative in assessment of the iron status in patients with mixed type anemia. In a study of 177 patients with selected chronic diseases [28], sTfR-F index showed 100% sensitivity and specificity, at a cutoff value of >1.62, to detect IDA. On the other hand there are reports showing that the calculation of the index did not improve the diagnostic accuracy of sTfR alone [29, 30]. In another large study with 337 anemic patients, a cutoff value of 2.5 for the sTfR-F index, showed 87% sensitivity and 96% specificity for predicting IDA, with an AUC 0.97, whereas the combination of this index with the reticulocyte hemoglobin equivalent provided the highest sensitivity and specificity [31]. Summarizing the results from three previous studies, it seems that sTfR-F has a sensitivity range from 50 to 94% and specificity range from 96.1 to 100%, with cutoff values from 1.2 to 2.5 [14,25,32].

One of the most important advantages of the sTfR-F index is that it is independent of the inflammatory status, also confirmed by our study, taking into account that no correlation was found between this index and CRP levels or disease activity. This is not unexpected, since in the literature there are no differences in the sTfR-F index between patients with ACD and HC, whereas differences between IDA groups and HC are statistically significant.

One limitation of our study is the rather small number of patients with ACD (4%) and mixed IDA/ACD (8%), which could decrease the detection rate of these patients, as far as all the indices are concerned. Moreover, the group of HC had a significantly higher ratio of male/female compared with the group of IBD patients. The higher number of females in IBD group could influence the results due to the possible influence of menstruation on the parameters of iron deficiency. Another limitation is the rather small number of patients with active disease not allowing for firm conclusions with respect to the role of disease activity on sTfR-F index.

Regarding sTfR measurement, it should be mentioned that there are certain disadvantages. The assay is not widely available; it remains expensive and is not standardized among different laboratories [33].

In conclusion, our results suggest that the sTfR-F index seems to be very efficient in the detection and diagnosis of IDA, among patients with IBD. Its detection rate is higher than sTfR alone and of course higher than the other existing markers. It is essential to mention that the clinical use of this index rather adds to the value of other established markers, such as ferritin, transferrin and Tsat, than replaces them in the diagnosis of IDA. Therefore, sTfR-F index could be proposed as an additional parameter, which can improve the diagnosis of iron deficiency anemia in patients with IBD. This remains to be confirmed with other larger studies.

Summary Box:

What is already known:

- No reliable biochemical markers exist for the differentiation between iron deficiency anemia (IDA) and anemia of chronic disease (ACD) in the setting of inflammatory bowel disease (IBD).
- There is evidence that soluble transferrin receptor (sTfR) is not influenced by chronic or acute inflammation.
- Literature data suggest that sTfR- ferritin (sTfR-F) index has a high diagnostic power in the diagnosis of IDA.

What the new findings are:

- High sTfR-F index has a better discriminating power than high sTfR levels in the diagnosis of IDA.
- sTfR-F index is a very efficient marker for the detection and diagnosis of IDA among patients with IBD
- sTfR-F index could be proposed as an addition to the established markers for the diagnosis of IDA in patients with IBD.

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