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The Role of Hepcidin, sTfR, and sTfR/Log Ferritin Index for the Differential Diagnosis of Iron Deficiency Anemia and Anemia of Chronic Disease

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Abstract

Background: In chronic diseases characterized by persistent inflammation, anemia of chronic disease (ACD) and iron deficiency anemia (IDA) are commonly encountered forms of anemia and can often co-occur. In such situations, the conventional iron status tests used for differential diagnosis are influenced by inflammation, reducing their diagnostic accuracy. The primary objective of this study was to assess the significance of hepcidin as a crucial diagnostic marker for ACD and to investigate the correlation between hepcidin and inflammation-related indicators. Furthermore, a significant secondary aim of this research was to ascertain the diagnostic role of novel biochemical markers, specifically soluble transferrin receptor (sTfR) and the derived sTfR/log ferritin index (sTfR-F index).

Methods and Results: This study was conducted at the Laboratory Service of the University Hospital Center «Mother Teresa» and included a cohort of 187 subjects, comprising 156 patients (83 females and 73 males) who were admitted to and received treatment at the Rheumatology and Cardiology Departments of the «Mother Teresa» University Hospital Center in Tirana. Additionally, 31 individuals without anemia and inflammatory conditions were included as a control group. All subjects incorporated into the study were classified into five distinct groups based on a comprehensive analysis of their complete blood profiles, iron status, and inflammation-related biomarkers: IDA, ACD, ACD+IDA, patients without anemia, and the control group without anemia.

The comparison between groups showed that the mean values of pro-hepcidin, TNFα, IL6, Hs-PCR, and ferritin are significantly decreased in IDA vs. ACD, while sTFR and sTfR-F index are significantly increased. In comparing ACD vs. ACD+IDA groups, ferritin increased significantly in the ACD group, while sTFR and sTfR-F index decreased significantly in ACD, compared to the ACD+IDA group. The ROC curves analysis of the biochemical parameters selected for the comparison of ACD vs IDA showed that the pro-hepcidin test is a perfect test for the differential diagnosis of ACD vs. IDA. The suggested cut-off value for pro-hepcidin was ≥153 ng/ml, yielding a sensitivity of 100% and a specificity of 100% for the diagnosis of ACD. As regards sTFR, the suggested cut-off value for the diagnosis of IDA vs. ACD was ≥ 4.9 µg/ml resulting in 84% sensitivity and 100% specificity. The sTfR-F index was also very useful for the diagnosis of IDA vs. ACD: for a cut-off value of ≥2.06, the sensitivity was 90% and the specificity was 96%. sTfR resulted in a good test for the diagnosis of ACD+IDA vs. ACD: the cut-off value was ≥ 3.7 µ/ml, the sensitivity was 92.3% and the specificity was 93.2%. Similarly, the parameter sTfR-F index resulted in a very good test for the diagnosis of ACD+IDA vs. ACD: for the suggested cut-off value of ≥2.3, the sensitivity was 92.3% and the specificity was 100%.

Conclusion: As a result of the study, it was found that the pro-hepcidin test was a highly accurate test for distinguishing between ACD and IDA. Meanwhile, sTfR and the sTfR-F index proved to be excellent indicators for the differential diagnosis of IDA in chronic inflammatory conditions.**(International Journal of Biomedicine. 2024;14(1):45-51.)**

Keywords: anemia of chronic disease • iron deficiency anemia • pro-hepcidin • transferrin receptor

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Abbreviations

ACD, anemia of chronic disease; **IDA**, iron deficiency anemia; **CHF**, chronic heart failure; **RA**, rheumatoid arthritis; **TfR**, transferrin receptor; **sTfR**, soluble TfR; **TfS**, transferrin saturation; **sTfR-F index**, sTfR/log ferritin index.

Introduction

Chronic infection, inflammation, and neoplastic pathology are commonly associated with anemia called "anemia of inflammation" or anemia of chronic disease (ACD). This form of anemia and iron deficiency anemia (IDA) are the most frequent forms of anemia encountered.[1,2] Various studies have reported the prevalence of ACD in chronic disease as 30%-70% in patients with rheumatoid arthritis (RA),[3,4] 28%-55% in patients with HIV infection (depending on the extent of the disease),[5] 30%-70% in patients with chronic liver disease[6] and 57% in patients with congestive heart failure.[7] In patients with malignant pathologies, the prevalence of anemia varies greatly depending on the stage of the disease and the therapy used. In most cases, the anemia of these pathologies has the characteristics of ACD.[8]

The pathophysiology of ACD during inflammation is intricately linked to the action of cytokines and reticuloendothelial system cells. These mechanisms result in various alterations, including the regulation of iron homeostasis, inhibition of erythroid precursor proliferation, attenuation of the erythropoietin response to anemia, and a reduction in the lifespan of erythrocytes. These modifications are predominantly mediated by hepcidin, recognized as the principal hormone governing iron balance.[9,10]

Inflammatory cytokines, particularly interleukin-6 (IL-6), are pivotal in stimulating hepatocyte hepcidin production.[11] Hepcidin functions by obstructing the functional activity of ferroportin, which serves as the primary conduit for iron export, leading to diminished absorption of dietary iron in enterocytes and reduced iron release into the bloodstream from splenic red pulp macrophages and hepatocytes. Consequently, this sequesters iron within the splenic red pulp macrophages, thereby decreasing iron availability for supporting erythropoiesis, even when iron stores are sufficient. This state is frequently referred to as «functional iron anemia.»[12]

As a distinctive feature of ACD, the serum typically displays normal or elevated ferritin levels, reflecting the accumulation and storage of iron within the reticuloendothelial system. Further elevations in ferritin levels occur due to immune activation.[13] The combination of reduced serum iron concentration and normal or elevated serum ferritin concentration is paramount in distinguishing ACD from IDA.[14]

In the context of chronic diseases, it is not uncommon to encounter not only ACD and IDA but also a mixed form of anemia known as ACD+IDA, particularly in cases of chronic inflammatory diseases that involve bleeding and/or malnutrition.[15,16]

The laboratory diagnosis of iron deficiency anemia in the presence of chronic inflammation poses significant challenges. This is because the acute phase of inflammation impacts transferrin saturation (TfS) and ferritin levels, making identifying an absolute iron deficiency quite challenging. Conversely, markers such as soluble transferrin receptor (sTfR), fragments generated through the proteolytic cleavage of the extracellular domain of transferrin receptor (TfR), directly reflect the functional iron status. Notably, sTfR is not influenced by acute inflammation, unlike ferritin, an acute-phase protein. Moreover, the sTfR/log ferritin index (sTfR-F index), calculated as the ratio of sTfR to the logarithm of ferritin, offers an even more accurate representation of the body's iron status.[17-19]

The primary objective of this study was to assess the significance of hepcidin as a crucial diagnostic marker for ACD and to investigate the correlation between hepcidin and inflammation-related indicators. Furthermore, a significant secondary aim of this research was to ascertain the diagnostic role of novel biochemical markers, specifically sTfR and the derived sTfR-F index. These markers were particularly evaluated for their diagnostic utility in distinguishing IDA and, more notably, in the differential diagnosis of IDA in chronic inflammatory conditions.

Materials and Methods

This study included a cohort of 187 subjects, comprising 156 patients (83 females and 73 males) who were admitted to and received treatment at the Rheumatology and Cardiology Departments of the «Mother Teresa» University Hospital Center in Tirana. Additionally, 31 individuals without anemia and inflammatory conditions were included as a control group. All patients were meticulously selected following a protocol that had obtained approval from the Biochemical-Clinical Laboratory Service in collaboration with the respective clinical departments.

Conforming to the guidelines set forth by the WHO, individuals were categorized as anemic if their hemoglobin levels fell below 12 g/dl for women and 13 g/dl for men. The subjects incorporated into the study underwent classification into five distinct groups based on a comprehensive analysis of their complete blood profiles, iron status, and inflammation-related biomarkers:

1. Patients with IDA: These individuals exhibited diminished sideremia levels and ferritin concentrations below 30 ng/ml.

2. Patients with ACD: This group presented reduced sideremia levels and manifested either elevated ferritin levels exceeding 100 ng/ml or ferritin levels ranging from 30 to 100 ng/ml, coupled with an sTfR-F index <1.

3. Patients with ACD+IDA: These patients exhibited diminished sideremia, alongside ferritin levels ranging from 30 ng/ml to 100 ng/ml, and notably, a sTfR-F index >2.

4. Patients suffering from RA or CHF without anemia: These subjects had been diagnosed with either RA or CHF, yet they did not present symptoms of anemia.

5. Control group without anemia: Comprising individuals who had undergone outpatient assessments, this group was characterized by the absence of anemia, as well as the presence of indicators denoting normal iron status and inflammation.

The prevalence of different forms of anemia in the patients with these chronic diseases included in the study was evaluated.

Statistical analysis was performed using the statistical software package SPSS version 21.0 (SPSS Inc, Armonk, NY: IBM Corp). Baseline characteristics were summarized as frequencies and percentages for categorical variables. Mean, standard deviation (SD), Median (Me), and 95% confidence interval (95% CI) were calculated. For comparisons between 2 independent groups, Student's t-test was applied. One-way ANOVA with the Tamhane post hoc test was used for multiple groups. Spearman's rank correlation coefficient was calculated to measure the strength and direction of the relationship between two variables. A probability value of $P<0.05$ was considered statistically significant.

Receiver Operating Characteristic (ROC) curves were employed to assess the sensitivity and specificity of selected laboratory parameters, namely hepcidin, sTfR, and the sTfR-F index, in the context of distinguishing between ACD and the IDA, as well as between ACD and ACD+IDA. Hepcidin was evaluated through the DRG Elisa prohormone hepcidin kit for the dosage of the prohormone hepcidin in serum, which correlates with the levels of hepcidin.

The sensitivity and specificity levels of each chosen laboratory parameter were meticulously documented. The Area Under the Curve (AUC) was calculated, serving as a metric to gauge the capacity of the respective laboratory parameter to accurately categorize patients with distinct forms of anemia. Various threshold values, or cut-offs, were identified for these laboratory parameters, and corresponding sensitivity and specificity values were reported for each cut-off. For the purposes of differential diagnosis, the most suitable cut-off values were determined based on clinical judgment.

Results

The study's findings revealed the presence of anemia in 45.7% of patients with RA, with 45.4% attributed to ACD, 29.5% to IDA, 20.4% to the mixed form ACD+IDA, and 4.5% to other forms of anemia. In patients with CHF, anemia was detected in 50.7% of cases, with 64.9% of those cases corresponding to ACD, 16.2% to IDA, 10.8% to ACD+IDA, and 8.1% to other forms of anemia.

Table 1 presents the data on the ages, genders, and laboratory test results of 156 patients and 31 healthy individuals. Table 2 compares laboratory parameters between different groups in the study. The Tamhane t-test was used according to the ANOVA post hoc procedure to compare continuous parameters between groups with unequal variances. The comparison between groups showed that the mean values of pro-hepcidin, TNFα, IL6, Hs-PCR, and ferritin are significantly decreased in IDA vs. ACD, while sTfR and sTfR-F index are significantly increased ($P<0.001$ in all cases). In comparing ACD vs. ACD+IDA groups, ferritin increased significantly in the ACD group, while sTfR and sTfR-F index decreased significantly in ACD, compared to the ACD+IDA group ($P<0.001$ in all cases).

The above data for the parameters of blood iron, ferritin, transferrin, TfS, sTfR, sTfR-F index, pro-hepcidin, and IL-6 are presented visually below by Error Bars graphics presenting the average values of the corresponding laboratory parameter in a 95% confidence interval (CI) for different groups of the study.

Table 1.

Gender, age and laboratories tests of 156 patients and 31 healthy individuals

Variable	The study groups				
	IDA (n=19)	ACD (n=44)	ACD+IDA (n=13)	PWA (n=75)	CG (n=31)
Male/ Female*	6/13	25/19	5/8	35/40	22/9
Age (years)^	54.7±12.0	58.6±13.2	59.8±14.0	56.6±11.7	58.6±11.9
RBC (×10⁶/μL)	4.3±0.3	4.0±0.6	4.1±0.4	4.6±0.5	4.9±0.4
Hb (g/dL)	10.2±1.4	11.2±1.4	11.3±1.3	13.8±1.3	14.4±1.3
HCT (%)	31.4±4.4	32.8±4.7	34.2±3.7	39.1±4.6	41.0±8.1
MCV (fL)	73.0±5.9	81.6±7.2	80.5±6.8	85.2±5.3	85.9±5.4
MCH (pg)	23.5±3.2	28.6±3.4	27.9±3.0	29.8±1.8	29.6±2.2
RET (/1000)	6.1±2.8	4.5±2.9	5.3±3.0	6.5±3.1	7.5±3.0
RDW (%)	16.2±1.6	14.0±1.1	15.2±1.4	13.9±1.7	14.1±1.3
Fe (μg/dL)	21.4±10.7	36.5±13.3	31.3±12.2	97.8±35.9	106.4±44.9
Ferritin (ng/mL)	17.6±12.4	270.7±211.2	55.5±19.4	132.4±82.7	140.9±83.4
Tf (mg/dL)	344.2±54.6	241.0±51.8	225.6±48.9	266.8±46.9	285.5±51.8
TFs (%)	3.5±2.8	8.2±5.7	7.0±5.0	21.3±14.2	26.0±9.6
sTfR (μg/mL)	7.9±4.0	2.2±1.1	7.0±2.8	2.1±1.0	2.1±0.9
sTfR-F index	7.8±4.9	0.9±0.5	4.2±1.9	1.1±0.6	1.1±0.5
TNFα (pg/dL)	5.9±1.6	13.9±4.7	12.9±6.7	6.1±1.7	5.6±1.9
Pro-hepcidin (ng/mL)	105.0±27.4	542.2±437.8	266.1±190.9	103.3±33.8	102.1±33.5
IL-6 (pg/mL)	3.8±1.0	16.8±10.0	11.2±6.1	3.9±1.4	3.4±1.2
Hs-PCR (mg/dL)	0.2±0.1	5.3±8.3	2.8±2.5	0.2±0.1	0.2±0.1
ESR (mm/hr)	20.3±15.8	43.1±12.0	38.1±5.2	17.5±14.8	10.2±5.0

**Gender presented as n/%; ^ Age and laboratories parameters presented as M ± SD; PWA- patients without anemia; CG -control group.*

Table 2.

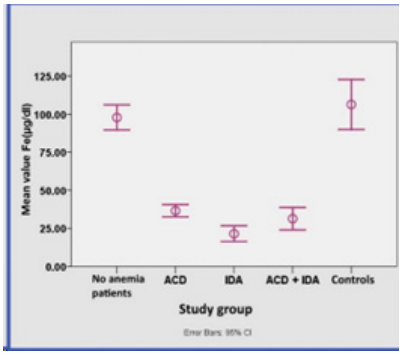
Laboratory parameters between different groups in the study.

Factor	Compared groups in the study					
	IDA vs. ACD	ACD vs. ACD+IDA	IDA vs. PWA	IDA vs. CG	ACD vs. PWA	ACD vs. CG
RBC (×10⁶/μL)	↑ 0.038	NS	↓ <0.031	↓ <0.001	↓ <0.001	↓ <0.001
MCV (fL)	↓ <0.001	NS	↓ <0.001	↓ <0.001	NS	↓ 0.047
MCH (pg)	↓ <0.001	NS	↓ <0.001	↓ <0.001	NS	NS
RDW (%)	↑ <0.001	NS	↑ <0.001	↑ <0.001	NS	NS
Fe (μg/dL)	↓ <0.001	NS	↓ <0.001	↓ <0.001	↓ <0.001	↓ <0.001
Ferr (ng/mL)	↓ <0.001	↑<0.001	↓ <0.001	↓ <0.001	↑ 0.001	↑ 0.005
Tf (mg/dL)	↑ 0.001	NS	↑ <0.001	↑ 0.006	NS	↓ 0.005
Tfs (%)	↓ 0.001	NS	↓ <0.001	↓ <0.001	↓ <0.001	↓ <0.001
sTfR (μg/mL)	↑ <0.001	↓ <0.001	↑ <0.001	↑ <0.001	NS	NS
sTfR-F index	↑ <0.001	↓ 0.001	↑ <0.001	↑ <0.001	NS	NS
TNF-α (pg/mL)	↓ <0.001	NS	NS	NS	↑ <0.001	↑ <0.001
Pro-Hep (ng/mL)	↓ <0.001	↑0.059	NS	NS	↑ <0.001	↑ <0.001
IL-6 (pg/mL	↓ <0.001	NS	NS	NS	↑ <0.001	↑ <0.001
HsCRP (mg/dL)	↓ 0.002	NS	NS	NS	↑ 0.002	↑ 0.002

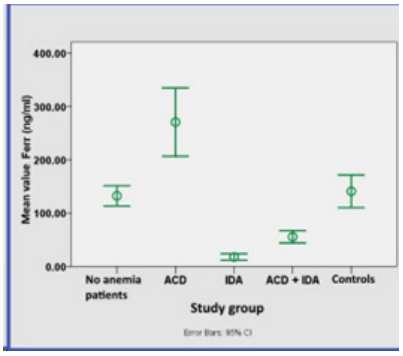
↓-Significantly lower compared to the group being compared

↑-Significantly higher compared to the group being compared;

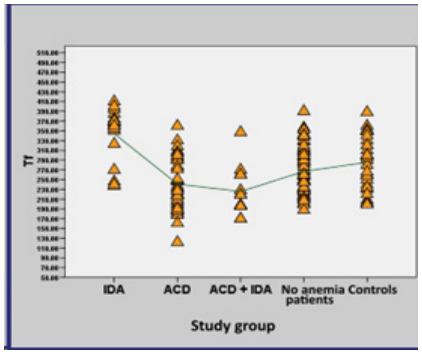
PWA- patients without anemia; CG -control group.



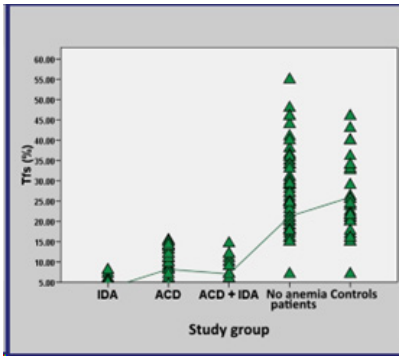
Graph 1. *Me±95% CI values of blood iron.*



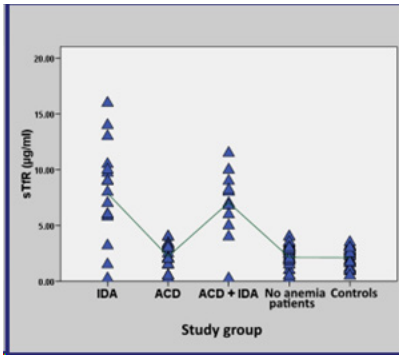
Graph 2. *Me±95% CI values of ferritin.*



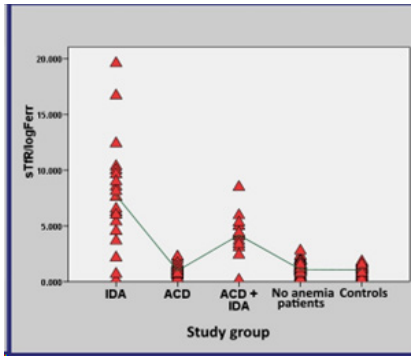
Graph 3. *Me±95% CI values of transferrin.*



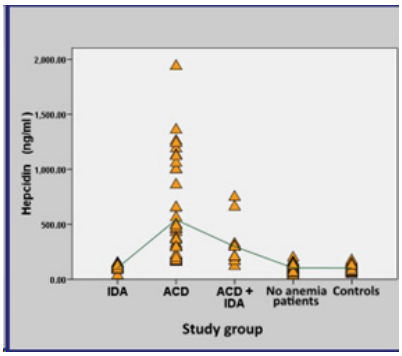
Graph 4. *Me±95% CI values of TfS.*



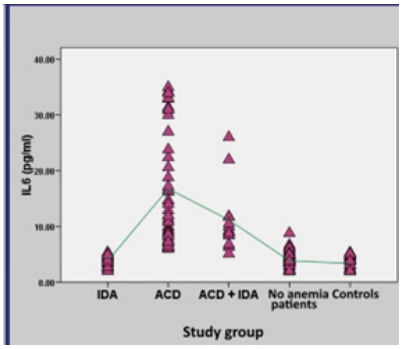
Graph 6. *Me±95% CI values of sTfR.*



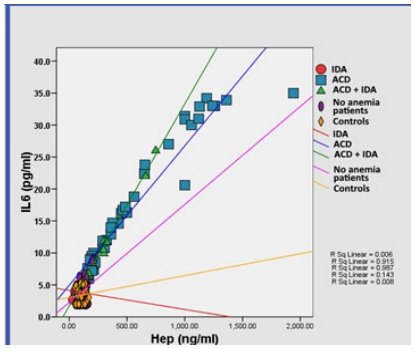
Graph 6. *Me±95% CI values of sTfR-F index.*



Graph 8. *Me±95% CI of pro-hepcidin.*



Graph 8. *Me±95% CI of IL-6.*



Graph 9. *The relationship between pro-hepcidin and IL-6.*

It can be clearly seen that for the ACD and ACD+IDA groups, the relationship between hepcidin and IL-6 is linear, while for the remaining groups it is not. The results of the linear regression confirm this: for patients with ACD, the increase in IL6 predicts the increase in pro-hepcidin for 91% of the cases. For patients with ACD+IDA, this reaches 98% (P<0.001).

The ROC curves analysis of the biochemical parameters selected for the comparison of ACD vs IDA showed that the pro-hepcidin test is a perfect test for the differential diagnosis of ACD vs. IDA. The suggested cut-off value for pro-hepcidin was ≥153 ng/ml, yielding a sensitivity of 100% (100% of those with ACD are correctly diagnosed and differentiated from those with IDA) and a specificity of 100% (all without ACD are correctly classified as without ACD). As regards sTFR, the suggested cut-off value for the diagnosis of IDA vs. ACD was ≥ 4.9 µg/ml resulting in 84% sensitivity and 100% specificity. The sTfR-F index was also very useful for the diagnosis of IDA vs. ACD: for a cut-off value of ≥2.06, the sensitivity was 90% and the specificity was 96%. sTfR resulted in a good test for the diagnosis of ACD+IDA vs. ACD: the cut-off value was ≥ 3.7 µ/ml, the sensitivity was 92.3% and the specificity was 93.2%. Similarly, the parameter sTfR-F index resulted in a very good test for the diagnosis of ACD+IDA vs. ACD: for the suggested cut-off value of ≥2.3, the sensitivity was 92.3% and the specificity was 100%.

Discussion

Anemia is a prominent symptom among hospitalized patients, and as discussed earlier, ACD and IDA are the most

prevalent forms of anemia in this context, which is further validated by the findings of our study.

Primarily, the evaluation of chronic disease-related anemia involves an assessment of iron status to exclude the possibility of IDA. In our study, we observed a decrease in both iron and saturated transferrin levels in the serum in both ACD and IDA when compared to the control group. (Graphs 1 and 2) This decline can be attributed to the complete absence of iron in IDA and iron sequestration within the splenic red pulp macrophages in ACD.[2,15,16,20]

Additionally, our study revealed that the levels of TfS in IDA patients are significantly lower than in ACD patients (P<0.001). This can be explained by the fact that concentrations of plasma iron transporter transferrin increase in IDA. However, other studies have also demonstrated that in ACD, the levels of transferrin, which acts as a negative antagonist of the acute phase, either remain normal or decrease due to the influence of inflammatory cytokines in this particular form of anemia.(1) When comparing TfS levels in ACD vs. ACD+IDA, the ANOVA test indicates that these differences are not statistically significant (P>0.05).

Retrospective studies[21] have previously shown that the transferrin concentration in IDA tends to be higher than in ACD, a finding that our study confirmed. However, it is worth noting that although the group of patients with ACD+IDA displayed variations in their transferrin concentrations, in comparison to the ACD group, most of these values still fell within the range of normal values when compared to those of healthy subjects (Table 1, Graph 3). Consequently, our results, consistent with the existing literature, lead us to conclude that the serum transferrin level does not provide a definitive indicator for detecting IDA in the presence of inflammation.

Ferritin, being a crucial iron status indicator that signifies iron storage, presents a subject of debate when it comes to determining a cut-off value for defining depleted iron reserves.[22,23] In various studies, this cut-off threshold varies, with some researchers adopting values as low as ≤12 ng/ml while others propose a higher threshold, such as ≤15 ng/ml. Additionally, due to ferritin's responsiveness as an acute-phase protein, some experts recommend even higher values like 22 ng/ml or 30 ng/ml.

Must et al.[24] found that a ferritin level below 12 ng/ml exhibits an excellent specificity of 98% but a low sensitivity of 20% in diagnosing IDA. In contrast, a cut-off value of 30ng/ml essentially transforms it into an almost flawless test for IDA diagnosis, boasting 100% sensitivity and 98% specificity. Other studies confirm this finding.

Patients who struggle with ACD while concurrently experiencing depleted iron reserves pose a particular challenge in the differential diagnosis of these two types of anemia. When we compared IDA to ACD and the control group, we observed that the serum ferritin concentration was significantly lower in IDA (P<0.001). Likewise, when comparing ACD to ACD+IDA and the control group, we noticed that the serum ferritin concentration was significantly higher in ACD than in ACD+IDA and the control group (P<0.001). In the case of patients with ACD, ferritin levels remain within the normal range or become elevated due to the influence of inflammation and the actions of inflammatory cytokines, which play a central role in the pathogenesis of ACD. Consequently, iron deposition and storage within the splenic red pulp macrophages are increased.[20,22] In the differential diagnosis between ACD and ACD+IDA, the cut-off value of ferritin for the diagnosis of IDA is calculated to be approximately 40-60 ng/dL.[23,25] Therefore, for ferritin values 30-100ng/dl, iron deficiency can neither be detected nor excluded based on the concentration of ferritin in the serum.

Based on the limitations of the above tests, which are influenced by the acute phase of inflammation, as well as the fact that sTfR concentrations are not influenced by acute-phase reactions, we decided to study the role of this biomarker, compared to other biomarkers of iron status, both in diagnosing pure IDA and in detecting IDA in conditions of inflammation.[22,26]

The mean and standard deviation of sTfR were evaluated in all groups included in the study, such as IDA, ACD, ACD+IDA, and patients without anemia, and the control group (Graph 5). Our data show that the serum concentration of sTFR in IDA presents high values when we compare this group with ACD and the control group (P<0.001); and it presents values within the normal limits in ACD when we compare this group with the control group. The value of sTfR 4.9 µg/ml serves as a cut-off to make the differential diagnosis between IDA and ACD (sensitivity 84% and specificity 100%). Also, when we compare ACD with the mixed form of anemia, we notice that the concentrations of sTfR in the serum are significantly lower in ACD, and the sensitivity and specificity of this indicator go toward a perfect test. Thus, the sTfR value >3.7 µg/ml serves as a cut-off (sensitivity 92.3% and specificity 93.2%) to make the differential diagnosis between ACD and ACD+IDA. The superiority of this test over other parameters influenced by the acute phase of inflammation is also confirmed by other authors.[23,27,28]

Since sTfR evaluates only functional iron and ferritin stores iron, i.e., static iron, studies have recommended a new index that evaluates the entire iron kinetics and improves the diagnostic efficiency of ferritin. This index was evaluated in our study, and calculations were made to find its value. This parameter did not lose its significance in all possible study groups (IDA vs. ACD, IDA vs. the control group, ACD vs. ACD+IDA). Therefore, this index is a very important indicator not only in diagnosing IDA but also in discovering its existence in the conditions of inflammation. As such, it is also useful to determine the patients expected to benefit from supplemental iron. The value of sTfR-F index >2.06 is that it has a sensitivity of 90% and a specificity of 96% when IDA is compared with ACD. While comparing ACD+IDA vs. ACD for values of sTfR-F index >2.3, the sensitivity and specificity of this test go toward a perfect test (92.3% and 100% respectively).

The scientific basis for the combined use of ferritin and sTfR is their different behavior toward reduced iron reserves. Serum ferritin concentrations are linearly related to reduced iron stores, but there is no cut-off concentration to indicate when a given patient's iron stores are so depleted that iron availability has become a limiting factor for erythropoiesis.

The opposite is the case with sTfR, which at this point corresponds to the increased concentration in the serum. These studies have shown that the sTfR-F index is the most efficient parameter for diagnosing ACD+IDA vs. ACD.[23,28]

The role of hepcidin in iron metabolism has now been made very clear by many prestigious papers and is widely accepted.[29,30] The results of our study are in support of the great role of hepcidin in the control of iron metabolism by removing it from the circulation toward the RE block, a fact which we verify with the high levels of ferritin in the serum of patients with ACD, compared to the control group (P<0.001). The data of our study also confirm the strong relationship between hepcidin and mediators of inflammation, such as IL-6 (Graph 9). Our data confirm the value of the pro-hepcidin test for the differential diagnosis of ACD vs IDA. The suggested cut-off from the respective ROC curve is pro-hepcidin ≥153ng/ml. For this given level, the sensitivity is 100% (100% of those with ACD are correctly diagnosed and differentiated from those with IDA), and the specificity is 100% (all without ACD are correctly classified as without ACD).

In conclusion, in patients with ACD, the serum concentration of pro-hepcidin shows a notable and significant increase, compared to patients with IDA, individuals without anemia, and healthy controls. Hepcidin emerges as a highly accurate diagnostic tool for differentiating ACD and IDA. Moreover, a robust positive correlation exists between the serum concentration of pro-hepcidin and IL-6. Additionally, the parameters sTfR and sTfR-F index demonstrate their effectiveness as excellent diagnostic tests for IDA in chronic inflammation.

Competing Interests

The authors declare that they have no competing interests.

References

1. Weiss G, Goodnough LT. Anemia of chronic disease. N Engl J Med. 2005 Mar 10;352(10):1011-23. doi: 10.1056/NEJMra041809. PMID: 15758012.
2. Means RT Jr. Recent developments in the anemia of chronic disease. Curr Hematol Rep. 2003 Mar;2(2):116-21. PMID: 12901142.
3. Peeters HR, Jongen-Lavrencic M, Raja AN, Ramdin HS, Vreugdenhil G, Breedveld FC, Swaak AJ. Course and characteristics of anaemia in patients with rheumatoid arthritis of recent onset. Ann Rheum Dis. 1996 Mar;55(3):162-8. doi: 10.1136/ard.55.3.162. PMID: 8712878; PMCID: PMC1010122.
4. Baer AN, Dessypris EN, Krantz SB. The pathogenesis of anemia in rheumatoid arthritis: a clinical and laboratory analysis. Semin Arthritis Rheum. 1990 Feb;19(4):209-23. doi: 10.1016/0049-0172(90)90001-v. PMID: 2181669.
5. Zon LI, Groopman JE. Hematologic manifestations of the human immune deficiency virus (HIV). Semin Hematol. 1988 Jul;25(3):208-18. PMID: 3043675.
6. Siciliano M, Tomasello D, Milani A, Ricerca BM, Storti S, Rossi L. Reduced serum levels of immunoreactive erythropoietin in patients with cirrhosis and chronic anemia. Hepatology. 1995 Oct;22(4 Pt 1):1132-5. doi: 10.1016/0270-9139(95)90620-7. PMID: 7557862.
7. Okonko DO, Van Veldhuisen DJ, Poole-Wilson PA, Anker SD. Anaemia of chronic disease in chronic heart failure: the emerging evidence. Eur Heart J. 2005 Nov;26(21):2213-4. doi: 10.1093/eurheartj/ehi509. Epub 2005 Oct 4. PMID: 16204265.
8. Groopman JE, Itri LM. Chemotherapy-induced anemia in adults: incidence and treatment. J Natl Cancer Inst. 1999 Oct 6;91(19):1616-34. doi: 10.1093/jnci/91.19.1616. Erratum in: J Natl Cancer Inst 2000 Mar 15;92(6):497. PMID: 10511589.
9. Theurl I, Aigner E, Theurl M, Nairz M, Seifert M, Schroll A, Sonnweber T, Eberwein L, Witcher DR, Murphy AT, Wroblewski VJ, Wurz E, Datz C, Weiss G. Regulation of iron homeostasis in anemia of chronic disease and iron deficiency anemia: diagnostic and therapeutic implications. Blood. 2009 May 21;113(21):5277-86. doi: 10.1182/blood-2008-12-195651. Epub 2009 Mar 17. PMID: 19293425.
10. Ganz T. Hepcidin, a key regulator of iron metabolism and mediator of anemia of inflammation. Blood. 2003 Aug 1;102(3):783-8. doi: 10.1182/blood-2003-03-0672. Epub 2003 Mar 27. PMID: 12663437.
11. Nemeth E, Rivera S, Gabayan V, Keller C, Taudorf S, Pedersen BK, Ganz T. IL-6 mediates hypoferremia of inflammation by inducing the synthesis of the iron regulatory hormone hepcidin. J Clin Invest. 2004 May;113(9):1271-6. doi: 10.1172/JCI20945. PMID: 15124018; PMCID: PMC398432.
12. Ganz T, Nemeth E. Iron imports. IV. Hepcidin and regulation of body iron metabolism. Am J Physiol Gastrointest Liver Physiol. 2006 Feb;290(2):G199-203. doi: 10.1152/ajpgi.00412.2005. PMID: 16407589.
13. Alvarez-Hernández X, Licéaga J, McKay IC, Brock JH. Induction of hypoferremia and modulation of macrophage iron metabolism by tumor necrosis factor. Lab Invest. 1989 Sep;61(3):319-22. PMID: 2788773.
14. Cartwright GE. The anemia of chronic disorders. Semin Hematol. 1966 Oct;3(4):351-75. PMID: 5341723.
15. Theurl I, Mattle V, Seifert M, Mariani M, Marth C, Weiss G. Dysregulated monocyte iron homeostasis and erythropoietin formation in patients with anemia of chronic disease. Blood. 2006 May 15;107(10):4142-8. doi: 10.1182/blood-2005-08-3364. Epub 2006 Jan 24. PMID: 16434484.
16. Brugnara C. Iron deficiency and erythropoiesis: new diagnostic approaches. Clin Chem. 2003 Oct;49(10):1573-8. doi: 10.1373/49.10.1573. PMID: 14500582.
17. Skikne BS, Punnonen K, Caldron PH, Bennett MT, Rehu M, Gasior GH, Chamberlin JS, Sullivan LA, Bray KR, Southwick PC. 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. 2011 Nov;86(11):923-7. doi: 10.1002/ajh.22108. Epub 2011 Aug 2. PMID: 21812017.

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18. Mast AE, Blinder MA, Gronowski AM, Chumley C, Scott MG. Clinical utility of the soluble transferrin receptor and comparison with serum ferritin in several populations. Clin Chem. 1998 Jan;44(1):45-51. PMID: 9550557.
19. Günther F, Straub RH, Hartung W, Fleck M, Ehrenstein B, Schminke L. Usefulness of Soluble Transferrin Receptor in the Diagnosis of Iron Deficiency Anemia in Rheumatoid Arthritis Patients in Clinical Practice. Int J Rheumatol. 2022 Oct 12;2022:7067262. doi: 10.1155/2022/7067262. PMID: 36275413; PMCID: PMC9581666.
20. Weiss G. Pathogenesis and treatment of anaemia of chronic disease. Blood Rev. 2002 Jun;16(2):87-96. doi: 10.1054/blre.2002.0193. PMID: 12127952.
21. Guyatt GH, Oxman AD, Ali M, Willan A, McIlroy W, Patterson C. Laboratory diagnosis of iron-deficiency anemia: an overview. J Gen Intern Med. 1992 Mar-Apr;7(2):145-53. doi: 10.1007/BF02598003. Erratum in: J Gen Intern Med 1992 Jul-Aug;7(4):423. PMID: 1487761.
22. Theurl I, Aigner E, Theurl M, Nairz M, Seifert M, Schroll A, Sonnweber T, Eberwein L, Witcher DR, Murphy AT, Wroblewski VJ, Wurz E, Datz C, Weiss G. Regulation of iron homeostasis in anemia of chronic disease and iron deficiency anemia: diagnostic and therapeutic implications. Blood. 2009 May 21;113(21):5277-86. doi: 10.1182/blood-2008-12-195651. Epub 2009 Mar 17. PMID: 19293425.
23. Punnonen K, Irjala K, Rajamäki A. Serum transferrin receptor and its ratio to serum ferritin in the diagnosis of iron deficiency. Blood. 1997 Feb 1;89(3):1052-7. PMID: 9028338.
24. Mast AE, Blinder MA, Gronowski AM, Chumley C, Scott MG. Clinical utility of the soluble transferrin receptor and comparison with serum ferritin in several populations. Clin Chem. 1998 Jan;44(1):45-51. PMID: 9550557.
25. Juncà J, Fernández-Avilés F, Oriol A, Navarro JT, Millá F, Sancho JM, Feliu E. The usefulness of the serum transferrin receptor in detecting iron deficiency in the anemia of chronic disorders. Haematologica. 1998 Aug;83(8):676-80. PMID: 9793248.
26. Bianco I, Mastropietro F, D'Asero C, Graziani B, Piergrossi P, Mezzabotta M, Modiano G. Serum levels of erythropoietin and soluble transferrin receptor in the course of pregnancy in non beta thalassemic and beta thalassemic women. Haematologica. 2000 Sep;85(9):902-7. PMID: 10980626.
27. Ferguson BJ, Skikne BS, Simpson KM, Baynes RD, Cook JD. Serum transferrin receptor distinguishes the anemia of chronic disease from iron deficiency anemia. J Lab Clin Med. 1992 Apr;119(4):385-90. PMID: 1583389.
28. Suominen P, Punnonen K, Rajamäki A, Irjala K. Serum transferrin receptor and transferrin receptor-ferritin index identify healthy subjects with subclinical iron deficits. Blood. 1998 Oct 15;92(8):2934-9. PMID: 9763580.
29. Fleming RE, Bacon BR. Orchestration of iron homeostasis. N Engl J Med. 2005 Apr 28;352(17):1741-4. doi: 10.1056/NEJMp048363. PMID: 15858181.
30. Delaby C, Deybach JC, Beaumont C. L'hepcidine et le métabolisme du fer [Hepcidin and iron metabolism]. Rev Med Interne. 2007 Jul;28(7):510-2. [Article in French]. doi: 10.1016/j.revmed.2007.03.009. Epub 2007 Apr 5. PMID: 17445953.