REVIEW

Serum transferrin receptor in diagnosis of iron deficiency

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Abstract

Objective: **The aim of the present study is to assess the diagnostic value of the serum transferrin receptor in distinguishing IDA (iron deficiency anemia) from ACD (anemia of chronic diseases) and combination of IDA and ACD (COMBI anemia) as compared to conventional laboratory tests of iron metabolism.**

Background: **Serum iron and serum ferritin are tests most commonly used for the detection of iron deficiency, however their values may be falsely changed. Serum fransferrin receptor (sTfR) has been introduced as a new tool, and its values are not affected by an increase in cytokine production in ACD patients.**

Methods: **In the retrospective study, 39 patients with IDA, 29 patients with ACD and 25 patients with COMBI, were evaluated using iron status tests including sTfR assay. The control group consisted of 33 healthy adults.**

Results: **Serum iron values in IDA, ACD and COMBI groups were not significantly different. Serum ferritin values distinguished IDA from ACD reliably but the diagnostic usefulness of ferritin measurements in ACD and COMBI patients is limited for their large variation breadth.**

Serum TfR concentrations were elevated in the vast majority of the IDA and COMBI patients and distinguished them from the ACD group.

The distinguishing of IDA from COMBI of the single basis of iron status is still difficult. However, the detection of iron deficiency in COMBI patients is very useful for the initiation of replacement therapy.

Conclusion: **We conclude that sTfR measurement is a valuable non-invasive tool for the diagnosis of iron depletion and an attractive supplement to more conventional laboratory tests in the detection of depleted iron stores. (Tab. 2, Fig. 3, Ref. 25.)**

Key words: iron-deficiency anemia, serum iron level, transferrin receptor.

Iron deficiency is one of the most frequent worldwide pathological conditions (1). In general its diagnosis is easy, however the difficulties arise in case of coincidence of iron deficiency and the acute phase reaction, or anemia associated with chronical diseases. The most commonly used tests include serum ferritin, serum iron, total iron binding capacity (TIBC), mean corpuscular volume (MCV) and reticulocyte count (2, 3). Serum ferritin is used as the most reliable indicator of iron deficiency. However, ferritin is one of the acute phase reactants and its concentration in serum is influenced by various clinical conditions. Its value may be falsely high due to recent infections, inflammatory diseases, liver diseases or starvation (4, 5). The examination of stainable iron in bone marrow is the gold standard so far. Although iron staining from bone marrow aspirate is a sensitive and accurate method, this procedure is invasive and difficult to perform in all patients (6).

There is an evident clinical need for non-invasive and sensitive means for the detection of iron deficiency, and in recent years, the serum transferrin receptor (sTfR) level has been introduced as a promising new tool for the diagnosis of iron depletion (6, 7, 8).

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Transferrin receptor (TfR) is a disulphide-linked dimer of two identical subunits, found on most cells and especially those with a high iron requirement, such as immature erythroid and malignant cells (9, 10). Its function is to internalize the absorbed iron into target cells. Serum transferrin receptor (sTfR) is produced by proteolysing the of TfR that circulates in plasma in a concentration that is proportional to the total cell mass TfR.

Serum TfR is increased in iron deficiency, unaffected by chronic diseases and is comparable to bone marrow aspirate (11).

We have investigated whether the sTfR assay could be used in order to differentiate iron deficiency anemia (IDA) from anemia of chronic disease (ACD) and to diagnose mixed anemia (COMBI).

Patients and methods

Patients: The investigated population consisted of ninety-three anemic adult patients, 68 females and 25 males, aged 18 to 95 years (mean, 61.8 years) admitted to the 1st Department of Internal Medicine in Bratislava who underwent the examination of sTfR and conventional laboratory tests of iron status.

The patients were assigned to one of three groups on the basis of the iron status and clinical data. Thirty-nine patients, 31 females, 8 males, (mean age, 53.6 years) who fulfilled the criteria of iron deficiency without other accompanying diseases were classified as having IDA. Twenty-nine anemic patients, 18 females, 11 males, (mean age, 64.7 years) were classified as having ACD. Twenty-five patients, 19 females, 6 males, (mean age, 67.0 years) who had signed acute or chronic blood loss together with an infectious disease, a chronic inflammatory disease (rheumatoid arthritis, inflammatory bowel disease, etc.) or nonhematological malignancy were included into the COMBI group. Hematological malignancies were excluded from the study, as some of them have been reported to be associated with an elevated sTfR regardless of iron status of the patients (12, 13).

Patients who suffered from hemolytic anemia or defined deficiency of vitamin B_{12} or folic acid were excluded from the study population, as these conditions might be associated with elevated sTfR levels.

The control group (CONTROL) consisted of thirty-three healthy, randomly selected 23 females and 10 males, aged 18 to 49 years (mean age, 26.7 years).

Samples and analytical methods: Blood was collected using Sarstedt tubes system (Nümbrecht, Germany): Monovette KE for blood count and S-Monovette for other determinations. Serum was separated by centrifugation at 3300xg for 10 min and immediately frozen at -20 °C until tested. The sTfR was measured by enzyme immunometric assay (IdeA sTfR kit, Orion Diagnostica, Orion, Finland) with the reference distribution of sTfR concentration 1.0 to 3.7 mg/l. Blood count was measured electronically by Sysmex SF - 3000 (TOA, Japan). Serum iron (reference range, 9.5 to 29.9 μmol/l for men, 8.8 to 27.0 μmol/l for women) was measured using Ferrozine method without deproteinization based on photometric colour test (IRON SYS-1, Roche Diagnostic Systems). Ferritin (reference range, 30.0–233.0 ng/ml for men, 6.0–81.0 ng/ml for premenopausal women, 14–186 ng/ml for menopausal women) was measured using a microparticle enzyme immunoassay technology (IMx system, Ferritin, Abbott) (14). Unsaturated iron binding capacity (reference range, 20.0–62.0 μmol/l) was measured using a Ferrozine method based on photometric colour test of chelate complex (UIBC, Roche Diagnostic Systems). The rate of transferrin saturation (reference range, 18.0 to 40.0 %) was calculated as (iron/TIBC - total iron binding capacity)x100.

Statistical analysis: Data were expressed as mean ± standard deviation (SD). All calculation procedures (correlation coefficient, Student's t-test) were carried out using the Excel for Windows software package and methodic instructions by ISO norms (15).

Results

The results for blood count and iron status markers for 93 anemic patients are summarized in Table 1. As neither ferritin nor sTfR concentrations differed significantly between male and female patients, the results have been analyzed without distinguising between male and female patients (Figs 1–3).

Tab. 1. Laboratory tests of iron status according to patient groups (values without distinction of men and women).

	CONTROL n=33	IDA n=39	ACD n=29	COMBI n=25
Hemoglobin g/l	141.0±9.4	93.7±2.04	99.6±1.56	89.6±1.93
MCV fl	89.5±2.75	77.2±7.09	89.1±5.67	83.6±10.1
Iron μmol/l	18.1±5.04	3.4±1.69	4.64±2.78	4.49±5.32
Ferritin ng/ml	57.1±60.26	14.0±23.6	345.0±327.0	105.2±201.2
Transferrin saturation %	31.3±9.85	5.18±2.84	12.7±7.44	11.0±16.9
sTfR mg/l	1.84±0.8	7.81±3.16	2.38±0.91	6.71±2.58

Results are mean±SD

CONTROL — control group of healthy subjects, IDA — iron deficiency anemia, ACD — anemia of chronic diseases, COMBI — patients with mixed anemia, MCV — mean corpuscular volume, sTfR — serum transferrin receptor.
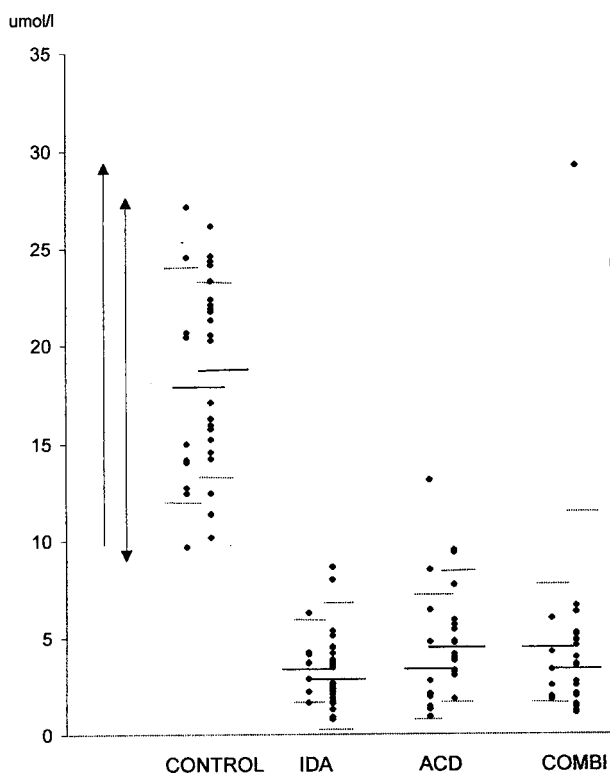
Fig. 1. Serum iron concentrations in control group and in anemic patients. Legend: means (solid lines) and standard deviations (dotted lines) are indicated, in each group, males are indicated in the first and females in the second column. ↑— serum iron reference range for men, ◊ — serum iron reference range for women, CONTROL — control group of healthy subjects, IDA — iron deficiency anemia, ACD — anemia of chronic diseases, COMBI — patients with combined IDA and ACD.
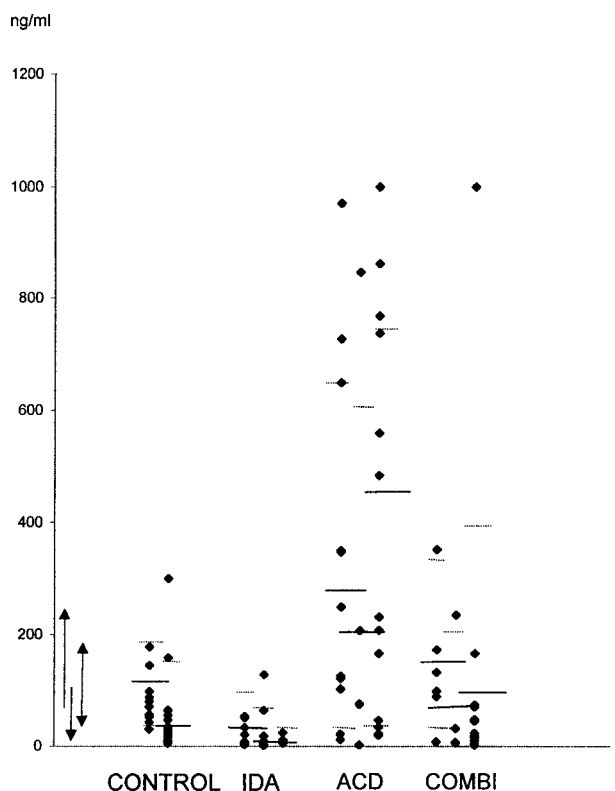

Figure 2. Serum ferritin concentrations in control group and in anemic patients. Legend: means (solid lines) and standard deviations (dotted lines) are indicated, in each group, males are indicated in the first column, premenopausal women in the second and menopausal women in the third column. ↑— ferritin reference range for men, ↓— ferritin reference range for premenopausal women, ◊— ferritin reference range for menopausal women, CONTROL — control group of healthy subjects, IDA — iron deficiency anemia, ACD — anemia of chronic diseases, COMBI — patients with combined IDA and ACD.

In IDA patients, the serum iron levels were situated below the reference range (mean±standard deviation [SD], 3.4±1.69 µmol/l) in 100.0 % and serum ferritin levels were situated below the reference range (mean±SD, 14.0±23.6 ng/ml) in 74.0 %. The sTfR levels were situated over the reference limit (mean±SD, 7.81±3.16 mg/l) in 95 %.

Patients with ACD had the serum iron levels below the reference range (mean±SD, 4.64±2.78 µmol/l) in 86 %. The ferritin levels were situated over the reference range (mean±SD, 345.0±327.0 ng/ml) in 55 %, and 34 % of levels were situated within the reference range for men and women. The sTfR levels were situated within the reference range (mean±SD, 2.38±0.91 mg/l) in 90 %, the others were higher (from 3.73 to 4.5 mg/l) in 10 %.

In COMBI group, the serum iron levels were situated below the reference range (mean±SD, 4.49±5.32 µmol/l) in 96 % and the ferritin levels (mean±SD, 105.2±201.2 ng/ml) were situated within the reference range in 56 %, the others were higher in 12 % and lower below the reference range in 32 %. The sTfR levels (mean±SD, 6.71±2.58 mg/l) were situated over the reference range in 96 %. The IDA serum iron levels did not differ

significantly from those of COMBI, as well as ACD from the COMBI group (Tab. 2, Fig. 1). However, serum iron was significantly higher in ACD than in IDA group (p<0.05).

The serum ferritin levels were significantly lower and the sTfR levels were significantly higher in patients with IDA (p<0.01, respectively) and COMBI (p<0.01, respectively) when compared to the patients with ACD (Tab. 2, Figs 2 and 3).

The sTfR levels in IDA patients did not differ significantly from those in COMBI. The ferritin levels were significantly higher in the COMBI group whem compared to those in the patients with IDA (p<0.05).

Discussion

Iron metabolism is complex, but can be viewed in the context of three pools: metabolic, transit and storage. Over 80 % of red blood cells and precursor cells constitute the metabolic pool. Red blood cell hem iron is measured as blood hemoglobin. When
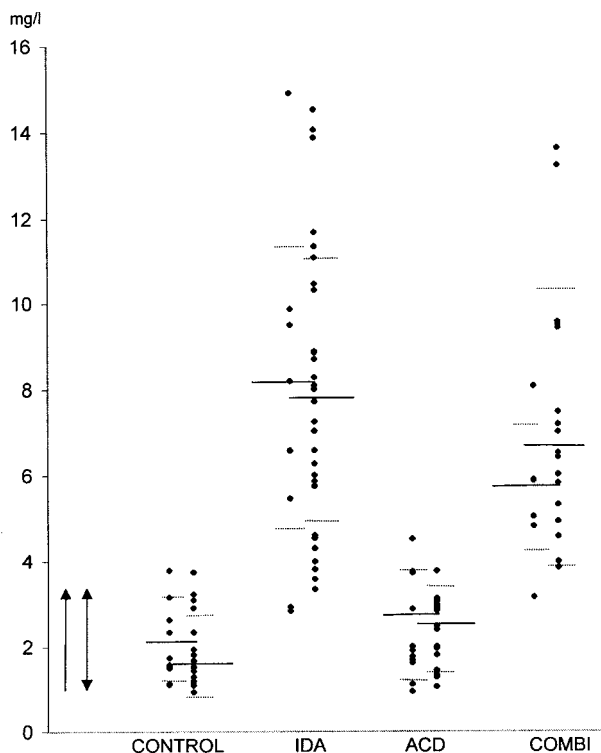
Fig. 3. Serum transferrin receptor concentrations in a control group and in anemic patients. Legend: means (solid lines) and standard deviations (dotted lines) are indicated, in each group, men are indicated in the first column and women in the second one. ↑— reference range for men, ◊ — reference range for women, CONTROL — control group of healthy subjects, IDA — iron deficiency anemia, ACD — anemia of chronic diseases, COMBI — patients with combined IDA and ACD.

hemoglobin is normal, the patient is less likely to be iron-deficient, but when it is not normal, iron deficiency is one of many possibilities. The transit pool can be measured directly as serum iron, serum transferrin and sTfR. The storage pool is measured by the concentration of serum ferritin that is roughly proportional to the total of iron stores. Iron deficiency is the most common cause of anemias, but ACD is more frequently in patients admitted to hospital. The recognition can be difficult but important, because when appropriate iron therapy may relieve the symptoms of anemia, however inappropriate therapy may lead to undesiderable side-effects. Iron deficiency may be a marker of a more sinister gastrointestinal pathology.

In our retrospective study, the patients were assigned to three groups on the basis of their iron status and clinical data. We did not use the staining of hemosiderin iron in bone-marrow aspirate, that is the clinical gold standard for assessing the iron status. It does not rank among basic tests because it is invasive, expensive and time-consuming, and the access to this test is limited for many physicians. The criteria used to classify the patients to different diagnostic categories (IDA, ACD, COMBI) dif-

fer from one study to an other, which makes the mutual comparison of studies difficult. Serum ferritin is an other problem as there is no agreement on the best lower reference limit, and its value increases in response to age and underlying inflammation (17).

The clinical situation in which serum transferrin receptor measurements have been suggested to be especially useful, is the differentiation of IDA from ACD (18). The distinction between IDA and anemia that accompanies infection, inflammation or malignancy is difficult, as the commonly used laboratory parameters do not necessarily distinguish these common causes of anemia. In the present study we have evaluated the clinical efficiency of the sTfR, serum iron and ferritin in the identification of patients with iron deficiency.

Serum iron did not distinguish IDA from ACD and COMBI groups. Its values were situated below the reference range in each of them (Tabs 1 and 2, Fig. 1). In the presented study, in anemic IDA patients without an accompanying infection, inflammatory or malignant disease, serum ferritin and sTfR values were significantly different from those in patients with ACD, but only 82 % of serum ferritin values were situated below the reference range in contrast to 95 % of sTfR values that were over the reference range (Tab. 2, Figs 2 and 3).

The diagnostic usefulness of ferritin measurements in ACD and COMBI patients may be reduced for the large variation breadth. In COMBI group, we observed low (28 %), normal (56 %) or high (16 %) serum ferritin values without the dependence on type of chronic diseases (Fig. 2). Serum TfR measurements with increased sTfR levels are able to distinguish ACD from COMBI patients more reliably (Tab. 2, Fig. 3).

The distinguishing of patients with IDA from those in the COMBI group is difficult for the large variation breadth of serum ferritin in the COMBI group and sTfR values that are situated over the reference range in both groups (Tables 1 and 2, Figs 2 and 3).

The results suggest that in COMBI patients, the potentially increased cytokine production does not greatly affect the TfR response caused by iron depletion. The detection of iron deficiency in the COMBI group is very useful for the initialisation of a targeted iron-replacement therapy and its monitoring.

Tab. 2. The statistical significance of differences (Student's t-test) of the iron status variables between the individual groups investigated.

	Serum iron	Ferritin	TfR
CONTROL vs IDA	p<0.01	p<0.01	p<0.01
CONTROL vs ACD	p<0.01	p<0.01	p<0.05
CONTROL vs COMBI	p<0.01	NS	p<0.01
IDA vs ACD	p<0.05	p<0.01	p<0.01
IDA vs COMBI	NS	p<0.05	NS
ACD vs COMBI	NS	p<0.01	p<0.01

NS — not significant, vs — versus, CONTROL — control group of healthy subjects, IDA — iron deficiency anemia, ACD — anemia of chronic diseases, COMBI — patients with mixed anemia.

It can be concluded from the results of our study, that low serum iron values are common in IDA, ACD and COMBI anemias. Low serum ferritin values and high sTfR values are found in IDA. Normal or high serum ferritin value together with normal sTfR value is found in ACD. Since serum ferritin values have a large variation breadth in COMBI anemias, it is difficult to differ these patients from those with ACD. On the other hand, the increased sTfR in COMBI anemias can help to distinguish this group from ACD more reliably.

The major advantage of sTfR measurements over serum ferritin is the apparent specificity of the biological response to changes in iron status and erythropoiesis. Thus, the sTfR measurement could serve as an attractive alternative to more conventional tests of iron status.

This study shows that serum TfR measurement is a useful tool in the differential diagnosis of iron deficiency anemias. Although, the combination of sTfR and ferritin measurements is very helpful in this condition, our results suggest that sTfR alone could be able to replace the conventional parameters of iron status in clinical laboratories.

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