Diagnostic relevance and correlations of soluble transferrin receptor in anaemia associated with chronic inflammatory diseases

Relevanța diagnostică şi corelații ale receptorului solubil pentru transferină în anemia din bolile inflamatorii

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Abstract

Hematopoietic progenitor cells, but also other rapidly dividing cell types express transferrin receptor, a molecule responsible for the uptake of iron from the circulating transferrin-Fe^{3+} complex. Due to proteolytic cleavage followed by shedding, a soluble form of the receptor (sTfR) appears in the bloodstream. sTfR has been proposed as a surrogate marker for evaluating iron stores and a diagnostic tool to differentiate between iron deficiency and anaemia of chronic disease.

We have studied the correlations of sTfR in 30 ferriprive anaemia patients with chronic inflammatory conditions (rheumatoid arthritis, spondylitis ankylopietica and osteoarthritis) with the main CBC parameters, CRP and serum iron levels. sTfR values have been compared with those of a gender- and age-matched healthy control group.

It has been proposed that sTfR is not modulated by inflammatory signals and therefore could substitute for transferrin and ferritin, very useful markers for iron deficiency, but influenced by the acute phase response. sTfR values were significantly higher in our anaemia patients than in control persons. Besides strong negative correlations with the main haematology parameters (Hgb, MCH, MCHC, MCV) we observed no relationship between sTfR and CRP. However, the existence of a negative correlation between WBC and sTfR has been demonstrated. In our opinion, these findings indicate that sTfR levels, which increase characteristically in iron depletion and in a lesser extent in ferriprive anaemia complicated with inflammation, could sometimes be insufficient in differential diagnosis of these two conditions.

***Key words**: soluble transferrin receptor, iron deficiency, anaemia of chronic inflammatory disease*

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Rezumat

Celulele progenitoare hematopoietice, dar şi alte tipuri de celule cu rată de divizie crescută exprimă receptorul de transferină, o moleculă care răspunde de preluarea fierului de la complexul circulant transferină-Fe $^{3+}$. Datorită clivării proteolitice şi năpârlirii ulterioare, forma solubilă a receptorului (sTfR) apare în sânge. S-a propus ca sTfR să fie aplicat ca marker surogat pentru evaluarea rezervelor de fier şi ca marker diagnostic de diferenţiere între depleţia de fier şi anemia asociată bolilor cronice.

S-au studiat corelaţiile receptorului solubil de transferină la 30 pacienţi cu anemie feriprivă asociată de afecţiuni inflamatorii cronice (poliartrită reumatoidă, spondilită anchilozantă, osteoartrită) cu parametrii hematologici principali, proteina C-reactivă, sideremia. Concentraţiile sTfR s-au analizat comparativ cu cele obţinute la o grupă de persoane fără anemie cu vârsta şi distribuţia sexelor asemănătoare.

Deoarece sTfR nu este modulat de semnalele inflamaţiei s-a propus că ar putea substitui transferina şi feritina, markeri importanţi în diagnosticul anemiei feriprive, dar influenţaţi de reacţia de fază acută. În studiul nostru, valorile sTfR au fost semnificativ mai crescute la pacienţii cu anemie decât la controli. Pe lângă corelaţiile negative dintre sTfR şi principalii parametri hematologici (Hgb, VEM, CMH, CMHE) am observat absenţa corelaţiei dintre receptorul de transferină şi PCR. Totuşi, s-a demonstrat existenţa unei corelaţii negative cu numărul leucocitar. Aceste rezultate, după opinia noastră, indică faptul că, determinarea nivelului sTfR, care creşte în mod caracteristic în depleţia de fier, şi în măsură mai mică în anemia feriprivă complicată cu răspuns inflamator poate fi uneori insuficient pentru diagnosticul diferenţial al condiţiilor menţionate.

Cuvinte-cheie: *receptorul solubil pentru transferină, deficit de fier, anemia din bolile inflamatorii cronice*

Introduction

The human transferrin receptor is a homodimeric membrane protein which mediates the cellular uptake of iron ions vehicled by transferrin. The ferri-transferrin/ transferrin receptor complex is internalized by chlatrin-mediated endocytosis, releases iron in the acidic environment of lysosomes and the apotransferrin/ transferrin receptor complex is recycled to the cell membrane (1). The soluble form of the receptor (sTfR) is generated by proteolytic cleavage between Arg100 and Leu101 via the action of an ADAM-type metalloproteinase (1,2). Structurally, the transferrin receptor can be divided into 2 domains: a short cytoplasmic domain and a larger, butterfly-shaped ectodomain possessing 3 subdomains: a protease-like portion, a helical structure interacting in dimerization and a carboxy-terminal, apical sequence (1).

Production of TfR in the cell is regulated by iron concentration via the action of a molecule called iron response/regulatory element binding protein (IRE-BP) (3). This protein binds to the hairpin like structure (IRE) in the 3' sequence of the transferrin receptor and also to the ferritin mRNA, inhibiting degradation of both RNA species (3).

Inside the cell, both TfR and sTfR bind to haemochromatosis protein (HFE), a direct regulator of iron concentration in macrophages. HFE mutations cause severe iron overload and progressive organ failure in the body (2). It has been postulated that sTfR may act as a modulator of iron export from enterocytes and macrophages and of iron storage in the liver (2).

Evidence has been provided that the proliferative rate of transferrin receptor –positive non-hematopoietic cells controls the plasma levels of sTfR and this receptor is actually overexpressed in different types of cancer (4-6).

Soluble transferrin receptor is also regulated hormonally by endogenous or exogenous sexual-steroids. T'Sjoen et al.(7) demonstrated that oral administration of ethynil-estradiol and androgen antagonist cyproterone acetate in male reduces sTfR with approximately 19% over 4 months, along with the decrease of hemoglobin and hematocrit. Since transferrin

receptor triggers apoptosis, it could become an attractive target for the development of therapeutic drugs in oncology (8).

Regarding clinical utility of sTfR measurement, it has been described as a good indicator of iron metabolism, although not very sensitive for the early or intermediate stages of iron deficiency; it is rather useful in the diagnosis of advanced iron deficiency anaemia (9). Serum sTfR correlates significantly with the reticulocyte count in healthy and with serum ferritin in patients with iron deficiency anaemia (10). Furthermore, levels of sTfR directly represent the overall mass of erythroid precursor cells, being considered an index of erythropoietic activity (11). While erythroid precursors are the source of about 80% of sTfR, other rapidly dividing cell types also contribute for the remaining 20% of the circulating sTfR quantities (2,12).

Currently, the widely accepted recommendations for using sTfR assays are:

- the differential diagnosis of iron deficiency anaemia and anaemia associated with chronic disease (10,13)
- the follow-up of the efficacy of recombinant human erythropoietin (r-Hu-EPO) treatment in patients with chronic renal failure (1,14, 15)

Differential diagnosis of iron deficiency and anaemia associated with chronic disease has usually been made by the assessment of bone marrow iron stores, an investigation that needs bone marrow biopsy and is not always accessible. Serum iron levels, transferrin and ferritin concentrations are important diagnostic marker molecules of ferriprive anaemia. However, because the levels of both ferritin and transferrin are influenced by the acute phase reaction (ferritin being increased while transferin is decreased), sTfR has been proposed as a surrogate marker for differential diagnosis of cases complicated with inflammatory conditions (13). sTfR remains in normal range in the course of anaemia associated with chronic disease, but iron deficiency anaemia is characterized by a 3-4 fold increase (10). Some studies provided the information that the sTfR/log ferritin ratio is superior to sTfR or ferritin alone in the assessment of iron depletion anaemia (11). Others stated that sTfR has a comparable value in differentiation of these two types of anaemia; moreover, it is very useful in the diagnosis of ferriprive status combined with acute phase reaction (13).

The reference values of sTfR have been stated by Raya et al. (16) in 885 healthy subjects (3-91 years) as follows (medians):

- 1.60 mg/l between 3-10 years,
- 1.42 mg/l between 11-20 years, males
- 1.33 mg/l between 11-20 years, females
- 1.16 mg/l in other age groups.

Since available data concerning the relationship of sTfR and inflammatory markers are contradictory (10,13,17), we targeted to investigate the correlations between sTfR and the main CBC (Complete Blood Cell count) parameters, with CRP, WBC in patients with anaemia of chronic inflammatory disease. Comparison with a group suffering of "pure" ferriprive anemia would have been interesting, but we did not include such a group since the gold-standard method to confirm ferriprive anemia (evaluation of bone marrow iron stores) was unavailable.

Materials and methods

We have determined the complete, 5-part differential blood cell count (CBC) - white blood cells (WBC), the absolute number of neutrophils, basophils, eosinophils, lymphocytes and monocytes, red blood cells (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), red cell distribution width (RDW), mean platelet volume (MPV) serum iron concentration, the serum levels of soluble transferrin receptor (sTfR) and high-sensitivity C-reactive protein (hsCRP) in 30 patients suffering of iron deficiency anaemia associated with chronic inflam-

matory conditions (13 patients with rheumatoid arthritis, 12 with spondylitis ankylopoetica and 5 with osteoarthritis) in comparison with 22 age- and sex-adjusted healthy controls without clinical and laboratory signs of anaemia.

Inclusion criteria for anaemia patients were haemoglobin<12g/dl and/or MCV<80 fl both for men and women, CRP>10 mg/l and/or WBC>9500/µl for inflammation. By gender, the patient group consisted of 17 women and 13 men, mean age ± std. error was 40.13 ± 2.76 years (13-62). In the control group 13 women and 9 men with a mean age of 42.15 ± 3.10 years (18-60) were enrolled.

Soluble transferrin receptor has been measured by an immunoturbidimetry method on KONE 300 automated biochemistry analyzer applying policlonal rabbit anti-TfR antibodies according to the recommendations of the manufacturer. Intra-assay variability of the test was 4.7%, inter-assay variability 5.5%. The complete blood count has been analyzed on a SYSMEX SF-3000 haematology analyzer. Serum iron levels have been assessed by a modified ortho-fenantrolin method applying spectrophotometry on a UVD-3200 UV-VIS instrument. Serum CRP has been detected by PEG-enhanced immunoturbidimetry (DIALAB reagents with policlonal goat anti-human CRP) on a COBAS MIRA biochemistry analyzer with the lower detection limit of 0.2 mg/l.

Statistical analysis have been performed with Excel 2003 and STATISTICA 5.0. Because the distribution of sTfR was abnormal, non-parametric tests have been applied; the level of statistical significance has been set to p=0.05.

Results and discussion

We investigated the distribution type for all parameters measured and found non-gaussian distributions (Kolmogorow-Smirnov and Lilliefors tests for normality) for sTfR, WBC, HgB, Htc, CRP. Serum transferrin receptor showed higher values in female than in male, but the difference did not reach statistical significance (4.06 ± 2.76 mg/l vs. 3.45 ± 2.33 mg/l, p=0.54). We could not observe any age-dependence in our anaemia patient group.

With the exception of WBC and platelets, the majority of CBC parameters were significantly different between the anaemia and the control group (*Table 1*). Mean values of the soluble transferrin receptor were more than 2.5 fold higher than the control group's values (3.91 ± 0.48 mg/l vs. 1.50 ± 0.12 mg/l, p<0.001, Mann-Whitney U test).

Considering a normal range of sTfR equalling mean ± 2 std. deviations derived from our control group, this could be defined as 0.4-2.6 mg/l. This interval shows an acceptable

Table 1. Comparison of the main haematological parameters of the two groups

Parameters	Anaemia patients (n=30)	Control group (n=22)	p value*
WBC (x 10^3)	10.22 ±1.31	7.88 ± 1.01	0.08
RBC (x 10^6)	4.00 ± 0.15	4.56 ± 0.08	0.003
Hemoglobin (g/dl)	10.192 ± 0.23	13.93 ± 0.16	<0.001
Hematocrit (%)	31.28 ± 0.68	41.33 ± 0.42	<0.001
MCV (fl)	76.75 ± 1.62	91.05 ± 1.27	<0.001
MCH (pg)	26.37 ± 1.00	30.66 ± 0.49	<0.001
MCHC (g%)	32.58 ± 0.26	33.66 ± 0.12	0.002
Platelets	258.77 ± 26.09	223.36 ± 10.59	0.48
sTfR (mg/dl)	3.91 ± 0.48	1.50 ± 0.12	<0.001

** Mann-Whitney U test*

Table 2. Correlations of sTfR in the anaemia patient's group

Correlations	Spearman R coefficient	p-level
sTfR & WBC	-0.16	0.41
sTfR & neutrophils	-0.13	0.49
sTfR & lymphocytes	-0.08	0.70
sTfR & monocytes	0.04	0.83
sTfR & eosinophils	0.02	0.92
sTfR & basophils	0.29	0.12
sTfR & RBC	0.29	0.13
sTfR & hemoglobin	-0.53	<0.01*
sTfR & hematocrit	-0.48	0.01*
sTfR & MCV	-0.39	0.05*
sTfR & MCH	-0.42	0.02*
sTfR & MCHC	-0.50	0.01*
sTfR & hsCRP	-0.16	0.49
sTfR & serum iron	0.25	0.20

concordance with the values indicated by Suominen et al. (18) 14 patients out of 30 presented sTfR values in this range, while 16 had higher sTfR concentrations. The same rate resulted when the cut-off value of 3.24 mg/l proposed by Choi et al. (9) has been set-up. In this interpretation, only 16/30 (53.3%) of our anaemia patients suffered of true iron deficiency, the rest being characterized by normal sTfR values associated with slightly higher hemoglobin concentration (10.29 ± 0.42 g/dl vs. 10.09 ± 0.41 g/dl) and MCV (79.43 ± 1.41 fl vs. 73.68 ± 2.72 fl, p=0.039) (*Figures 1, 2*).

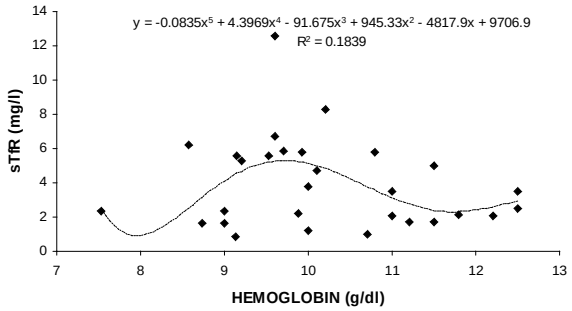

Figure 1. Correlation between sTfR and hemoglobinemia in iron deficiency anemia of inflammatory origin.

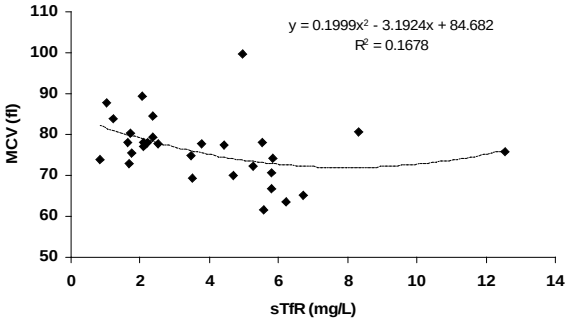

Figure 2. Correlation of sTfR and mean corpuscular volume in iron deficiency anemia with inflammation

Concerning the correlations of serum transferrin receptor, we found that significant negative correlations exist between sTfR and hemoglobin, blood hematocrit, MCH, MCH and MCHC. The strongest relationship could be stated between sTfR and MCHC (R = -0.50, p=0.01) (*Figure 3*). We observed the absence of correlation between circulating transferrin receptor and the absolute number of white blood cells, neutrophils, lymphocytes or monocytes.

Spearman correlation coefficients and p values for these parameters are listed in *Table 2*.

sTfR and serum iron concentrations showed a slightly positive trend lacking statistical significance. When serum iron concentrations have been divided into tertiles (lower tertile: Fe<9.34 μmol/l, upper tertile Fe>16.43

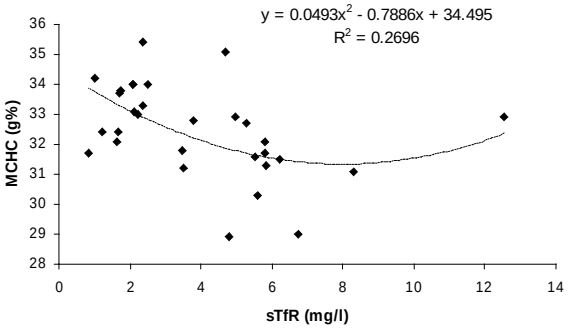

Figure 3. Correlation between soluble transferrin receptor levels and MCHC in iron deficiency anemia with inflammation

Table 3. sTfR values by WBC, NEU and CRP tertiles

sTfR (mg/l)	**WBC lower tertile (≤ 6730/µl)** 5.17 ± 0.96	**WBC upper tertile (≥ 9680/µl)** 3.09 ± 0.76	**p value** 0.043*
sTfR (mg/l)	**Neutrophil lower tertile (≤ 3620/µl)** 3.35 ± 0.68	**Neutrophil upper tertile (≥ 7430/µl)** 2.74 ± 0.56	NS
sTfR (mg/l)	**CRP lower tertile (≤13.31 mg/l)** 4.59 ± 1.14	**CRP upper tertile (≥ 42.24 mg/l)** 2.94 ± 0.62	NS

** p - Mann-Whitney U test*

µmol/l), the upper tertile showed lower CRP values (50.52 ± 14.22 mg/l vs. 24.24 ± 8.44 mg/l) and higher sTfR levels (4.92 ± 1.13 mg/l vs. 3.26 ± 0.59 mg/l) than the lower tertile, but these differences again did not reach statistical significance (*Figure 4*).

Despite of the fact that we could not establish direct correlations between soluble transferrin receptor levels and the total number of white blood cells or serum concentration of C-reactive protein, analyzing WBC and CRP by tertiles, each upper tertile showed lower sTfR values than the lower one. In the case of WBC, the difference was significant as it is shown in *Table 3*.

Performing the comparison of sTfR in the neutrophil tertiles, a non-significant difference (3.35 ± 0.68 mg/l vs. 2.74 ± 0.54 mg/l, p=0.57) resulted in the favour of the lower tertile (<3620/µl) vs. the upper tertile (>7430/µl).

Conclusions

The choice of iron supplementation in anaemia depends on the diagnostic accuracy of iron deficiency. While in ferriprive anaemia it is the only effiecient therapy, administration of oral or parenteral iron can even be harmful in anaemia of chronic disease, where iron depletion is apparent due to the increased phagocyt-

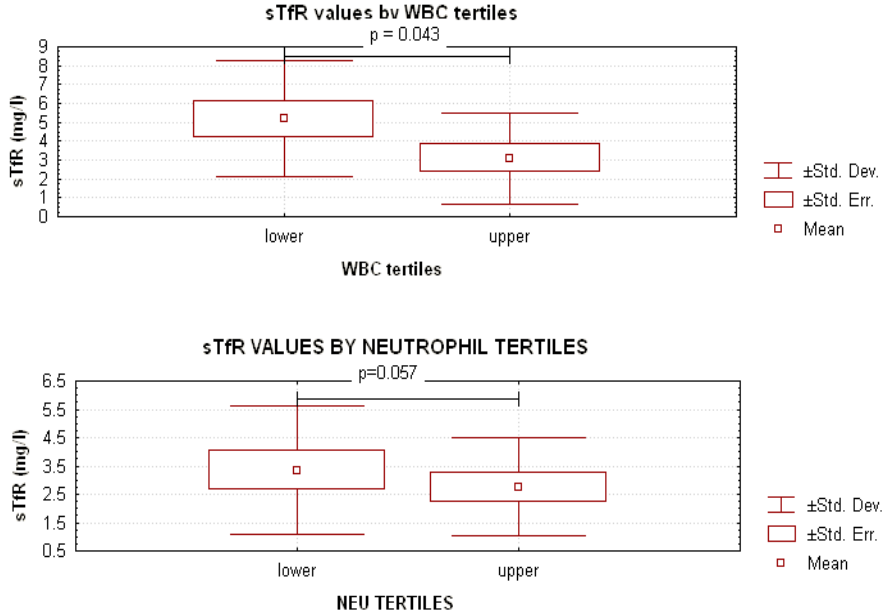

Figure 4. sTfR levels by the WBC and neutrophil upper vs. lower tertiles in patients.

otic activity of macrophages. Since transferrin is down-regulated and ferritin is up-regulated by the inflammatory pathway, soluble transferrin receptor was recommended by a number of authors to distinguish between simple iron deficiency and iron deficiency combined with chronic inflammation or infection (10,13).

Our results still do not answer the question whether combined application of sTfR and hsCRP enhances or not the diagnostic sensitivity. While others could establish significant negative correlation between these two markers (17), in our study only a weak negative trend could be observed. However, the negative correlation between sTfR and WBC may indicate a dependence from inflammatory signals. In our study, performed on a combined iron deficiency anaemia and acute phase reaction patient cohort, sTfR was increased and showed significant negative correlations with the main hematological parameters (hemoglobin, hematocrit, MCV, MCH, MCHC). Almost 50% of patients showed "normal" sTfR values suggestive for anaemia of chronic disease without overt acute phase reaction, but high CRP levels were contradictory to this finding. In our interpretation, this means that the determination of sTfR alone could lead to misdiagnosis of combined anaemia. sTfR solely may probably be helpful in distinguishing between true ferriprive anaemia and anaemia caused by chronic inflammation in selected patients, but determination of CRP is desirable in order to define exactly the extent of acute phase response.

Abbreviations

sTfR - soluble transferrin receptor
CRP – C-reactive protein
MCV - mean corpuscular volume
MCH - mean corpuscular hemoglobin
MCHC - mean corpuscular hemoglobin concentration
Hgb – haemoglobin
WBC – white blood cells
CBC – complete blood count

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