Relation between Level of Soluble Transferrin Receptor and Anemia of Chronic Kidney Disease

Nancy Esam Abdelaleem, Wafaa Elsaeed, Mai Abdelfatah
Department of Pediatrics, Faculty of Medicine, Zagazig University, Egypt
Corresponding Author: Nancy Esam Abdelaleem
Email: nancywaly123@gmail.com

Abstract

Background: CKD is defined as abnormalities of kidney structure or function, present for >3 months. Diagnostic thresholds for glomerular filtration rate (GFR) of less than 60 mL/min per 1.73 m² and an albumin–creatinine ratio (ACR) of 30 mg/g or greater were retained. In addition, childhood CKD registries are usually restricted to small reference populations, The median incidence of RRT (renal replacement therapy) in children < 20 years old is 9 per million of age-related population (pmarp) worldwide, and the prevalence is reported as 65 pmarp. The incidence of CKD also in Europe is reported to be around 11–12 per million of age-related population for stages 3–5. Anaemia is more prevalent with advancing stages of CKD. Data from the North American Pediatric Renal Trials and Collaborative Studies (NAPRTCS) show that the prevalence of anaemia in children is 73% at CKD stage III, 87% at stage IV and >93% at stage V. The transferrin receptor is a transmembrane cellular protein primarily expressed in cells that require iron, and the soluble form is elevated in serum and plasma in cases of iron deficiency. Serum ferritin levels reflect iron while sTfR levels reflect the degree of availability of iron for cells. Calculating the sTfR/log ferritin index (sTfR Index).

Keywords: Anemia, Chronic Kidney Disease, Soluble Transferrin Receptor

Background

CKD is defined as abnormalities of kidney structure or function, present for >3 months. Diagnostic thresholds for glomerular filtration rate (GFR) of less than 60 mL/min per 1.73 m² and an albumin–creatinine ratio (ACR) of 30 mg/g or greater were retained (1). Comparable amounts for other western countries, prevalence of the disease has significantly increased with the improvement of survival and treatment of CKD. Moreover, higher values for incidence and prevalence have been reported in the USA, because RRT is started earlier, at higher levels of GFR, in comparison with other developed countries (2). Anemia is a common complication in children with CKD causing many consequences, including poor quality of life, depressed neuro cognitive ability, exercise capacity and increase cardiovascular risk factors, such as left ventricular hypertrophy (LVH) (KDOQI, 2006). The National Kidney Foundation KDOQI (NFK-KDOQI) clinical practice guidelines use reference data from National Health and Nutrition Examination Survey (NHANES) to define normal values in the pediatrics population and recommend initiating an evaluation for anemia when hemoglobin levels fall below the age-specific and sex-specific 5th percentile value (1).
Anemia is more prevalent with advancing stages of CKD. Data from the North American Pediatric Renal Trials and Collaborative Studies (NAPRTCS) show that the prevalence of anemia in children is 73% at CKD stage III, 87% at stage IV and >93% at stage V (3).

Anemia of CKD is the result of many factors, but decreased production of erythropoietin and iron dysregulation (including iron deficiency and iron-restricted erythropoiesis) are the primary defects (4). The use recombinant human erythropoietin (rHuEPO) is safe and effective, both in children with conservatively treated CKD and in those on maintenance dialysis. The goal of this treatment is to achieve target hemoglobin levels of approximately 11 g/dL or slightly greater, hemoglobin levels >13 g/dL are not associated with improved patient outcomes in both adult and children (5).

Anemia is a common complication in children with CKD causing many consequences, including poor quality of life, depressed neuro cognitive ability, exercise capacity and increase cardiovascular risk factors, such as left ventricular hypertrophy (LVH) (KDOQI,2006). The National Kidney Foundation KDOQI (NFK-KDOQI) clinical practice guidelines use reference data from National Health and Nutrition Examination Survey (NHANES) to define normal values in the pediatrics population and recommend initiating an evaluation for anaemia when haemoglobin levels fall below the age-specific and sex-specific 5th percentile value (1).

Anemia is more prevalent with advancing stages of CKD. Data from the North American Pediatric Renal Trials and Collaborative Studies (NAPRTCS) show that the prevalence of anaemia in children is 73% at CKD stage III, 87% at stage IV and >93% at stage V (3).

Anaeimia of CKD is the result of many factors, but decreased production of erythropoietin and iron dysregulation (including iron deficiency and iron-restricted erythropoiesis) are the primary defects (3). The use recombinant human erythropoietin (rHuEPO) is safe and effective, both in children with conservatively treated CKD and in those on maintenance dialysis. The goal of this treatment is to achieve target haemoglobin levels of approximately 11 g/dL or slightly greater, haemoglobin levels >13 g/dL are not associated with improved patient outcomes in both adult and children (5).

**Soluble transferrin receptor**

Iron transport in the plasma is carried out by transferrin, which donates iron to cells through its interaction with a specific membrane receptor. The transferrin receptor (TfR) is a 760-amino-acid glycoprotein. The functional receptor is composed of two such monomers, linked by two disulfide bridges to form a molecule of 190,000 Da. Virtually all cells, except mature red cells, have TfR on their surface, but the largest numbers are in the erythron, placenta and liver. In a normal adult, about 80% of TfR are in the erythroid marrow (6).

Receptor density on proliferating cells is related to the availability of iron as deprivation of iron results in prompt induction of TfR synthesis whereas excess iron suppresses TfR numbers. Therefore, the total mass of cellular TfR depends both on the number of erythroid precursors in the bone marrow and on the number of TfR per cell, a function of the iron status of the cell. A circulating form of TfR has been found in human as well as animal serum. Serum TfR (sTfR) is a soluble truncated monomer of tissue receptor, lacking its first 100 amino acids, which circulates in the form of a complex of transferrin and its receptor (7).

A possible conformation is two receptor monomers (85 kDa) binding to one transferrin molecule (80 kDa) to give a total MW of around 250 kDa. A very small amount (although this may vary with the patient’s diagnosis) of circulating TfR is in the formof an intact dimer in exosomes. Soluble TfR is produced by proteolysis, mediated by a membrane associated serine protease that occurs mostly at the surface of
exosomes within the multivesicular body prior to exocytosis. The bulk of sTfR measured in serum is proportional to the mass of cellular TfR and originates mostly from erythroblasts and to a lesser extent from reticulocytes (8).

**Soluble TfR in normal subjects**

To measure sTfR quantitatively in human serum, respectively. A number of quantitative assays have been set up to measure sTfR levels in biological fluids such as culture supernatants and plasma or serum. Some have been developed in research laboratories but several are now commercially available. The performance of these assays is highly variable, but the major problem is the lack of an international standard (9).

Therefore, although levels measured by various assays may correlate well, direct clinical comparison of values obtained with different assays is difficult. sTfR levels averaged 5.0 ± 1.0 mg/l (mean F S.D.) in a group of 165 normal human subjects and were remarkably stable over time in the same subject (8).

No differences are observed with sex or age in the 18–80-year age range, but levels are somewhat higher in fetuses and newborns compared to adults. In the early postnatal period, sTfR levels are comparable to those of adults with marrow suppression, but they soon increase again so that infants and adolescents tend to have slightly higher sTfR levels than adults. Age-specific reference limits for children aged 0–4, 4–10 and 10–16 years could be derived in a carefully selected group of healthy children from the observation of a continuous decline of sTfR values from birth to late adolescence (9).

**Soluble TfR and pathophysiology of anemias**

Erythropoietin (Epo) production is regulated through a feedback system between the bone marrow and the kidney through which serum Epo levels increase exponentially in proportion to the degree of anemia. In the presence of a normal marrow stem cell reserve, erythropoiesis increases in proportion to Epo stimulation and thus sTfR also rise exponentially in response to anemia (10).

Reference regressions representing the normal relationships between hematocrit (Hct) on the one hand, and Epo or sTfR on the other, based on normal subjects and patients with hemolytic anemia. Based on these formulas, predicted log (Epo) and log (sTfR) values were derived for each Hct, O/P ratios of observed/predicted log (Epo) or log(sTfR) were derived, and 95% confidence limits were obtained in order to define a range of reference values for individual O/P ratios. These limits are 0.80–1.20 for O/P Epo and 0.90–1.10 for O/P sTfR (11).

The pathophysiology of anemia can therefore be assessed by a simple determination of the patient’s Hct, serum Epo and sTfR. These measurements are best expressed in relation to the degree of anemia to avoid gross overestimation of marrow proliferative capacity. When investigating a group of patients, this can be achieved by comparing patients and reference subjects (hemolytic anemia) by regression analysis. (10). When studying an individual patient, this can be obtained by the O/P ratio. Then not only the absolute rate but also the adequacy of Epo production (O/P Epo) and of marrow response to Epo stimulation (O/P sTfR) can be evaluated as appropriate or not for a given degree of anemia. As shown in Table 13, three major patterns of erythropoiesis are described on the basis of the Hct, O/P sTfR and O/P Epo, while the retic index and sTfR levels serve to separate major patterns into variants. Normal Hct, O/P Epo and O/P TfR define a normal pattern of erythropoiesis. The sTfR value helps differentiate a variant of compensated hemolysis in which sTfR is increased. Decreased Hct and normal O/P Epo and O/P sTfR characterize a pattern of “hyperdestruction”. The reticulocyte index helps break this group down into two variants of “hemolysis” (index >3) and “ineffective erythropoiesis” (10).
Figure (1). Mean ± S.D. sTfR levels in groups of patients with hyperplastic erythropoiesis, including autoimmune hemolytic anemia (AIHA), hereditary spherocytosis, thalassemia major (Thal), HbH disease (HbH), or polycythemia (PC). The gray zone represents the reference interval in normal subjects (10).

Table (1): Patterns of erythropoiesis in the investigation of the pathophysiology of anemia based on the hematocrit, the reticulocyte index, serum sTfR, O/P (observed/predicted) sTfR and O/P Epo

<table>
<thead>
<tr>
<th>Erythropoiesis pattern</th>
<th>Main characteristics</th>
<th>Hct</th>
<th>O/P sTfR</th>
<th>O/P Epo</th>
<th>sTfR</th>
<th>Retic indexa</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>Normal</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>Normal</td>
</tr>
<tr>
<td></td>
<td>Compensated</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>Hereditary spherocytosis</td>
</tr>
<tr>
<td>Hyperdestruction</td>
<td>Hemolysis</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>↑</td>
<td>AIHA</td>
</tr>
<tr>
<td></td>
<td>Ineffective</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>↑</td>
<td>Thalassemia</td>
</tr>
<tr>
<td>Hypoproliferation</td>
<td>Marrow failure</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td></td>
<td>Aplastic anemia</td>
</tr>
<tr>
<td></td>
<td>Defective Epo</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td></td>
<td>Renal failure</td>
</tr>
<tr>
<td>Mixed disorder</td>
<td>Marrow failure</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>↑</td>
<td>MDS</td>
</tr>
<tr>
<td></td>
<td>Defective Epo production</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td></td>
<td>Cancer</td>
</tr>
</tbody>
</table>

AIHA= autoimmune hemolytic anemia. MDS= Myelodysplastic syndrome. A Retic index: < 2 is low, > 3 is high. (index < 2). The pattern of “erythroid marrow hypoproliferation” is defined by erythropoietic activity inadequately low for the degree of anemia (decreased O/P sTfR) (12).
This can be related to defective Epo production (low O/P Epo) or intrinsic marrow failure (normal O/P Epo). Marrow hypo-proliferation can be absolute (decreased sTfR), i.e., “hypo-proliferative anemia”, or relative (normal/increased sTfR), termed “mixed disorder of erythropoiesis”, i.e. with both hyperdestruction and hypoproliferation components. The model is thus very useful for detecting the presence of multiple mechanisms of anemia in the same patient, particularly a component of hypoproliferation being associated with hyperdestruction (12).

This functional classification of anemia reveals that a same clinical diagnosis may be associated with different patterns of erythropoiesis, while a same pattern of erythropoiesis can be seen across different clinical groups. A typical example is the anemia of chronic disorders. Children with acute leukemia or solid tumors have adequate Epo production but profoundly suppressed erythropoiesis. Patients with chronic lymphocytic leukemia (CLL) appear to have appropriate Epo production and erythroid marrow response for the degree of anemia, although erythropoiesis may become impaired in advanced stages (12).

A significant proportion of multiple myeloma patients has relative marrow failure in large part due to inadequate Epo production. Defective iron supply to the erythron rather than blunted Epo production is the major cause of anemia associated with systemic onset juvenile arthritis, resulting in elevated sTfR levels that return to normal following correction of anemia with intravenous (IV) iron (13).

**Soluble TfR and iron status**

The iron status also influences sTfR levels in serum (Figure 7). In subjects with elevated transferrin saturation, African iron overload or genetic hemochromatosis, average sTfR levels are about 20% below those measured in normal subjects but most values are still within the normal range. Iron deficiency has a much stronger impact on sTfR levels. Soluble TfR levels in severely anemic iron-deficient rats increase many folds over normal values, in proportion to the increase documented for erythron membrane receptors (14).

As compared to normal individuals, levels are marginally increased in nonanemic iron-deficient subjects but more dramatically so in patients with iron deficiency anemia. In such patients, sTfR levels exhibit strong correlations with various red cell indices indicative of iron deficiency and are inversely related to serum ferritin. The elevation of sTfR with iron deficiency has been confirmed by the evaluation of stainable marrow iron (15).

![Figure 2](image)

**Figure (2):** Mean ± S.D. sTfR levels in groups of patients with abnormal iron status, including nonanemic iron deficiency (ID), iron deficiency anemia (IDA) and idiopathic hemochromatosis (IH) (16).
This indicates that iron-deficient erythropoiesis may occur in subjects thought to be iron replete on the basis of ferritin values. The normal values of sTfR, ferritin and sTfR–ferritin index should therefore be those obtained in these healthy adults after 3 months of iron supplementation had corrected subclinical iron depletion. In particular, the upper limit of normal sTfR values should be lowered to allow detection of incipient iron-deficient erythropoiesis. Iron stores may not need to be exhausted to have clinically relevant storage depletion. Subtle changes in functional iron supply have been illustrated in a study of obese patients undergoing a very-low-energy all-protein diet (11).

The serum iron and transferrin saturation fell sharply within 1 week and this was followed by moderate increases in sTfR correlating inversely with prior changes in serum iron. These results indicate that depressed serum iron, probably related to altered iron release by reticuloendothelial cells, induces functional tissue iron deficiency too short in duration to produce alterations in red blood cell indices. However, sTfR levels remained in the normal range and iron-deficient erythropoiesis could not have been detected if serial samples had not been obtained (17).

**Soluble TfR and inflammation**

As sTfR levels are not increased in patients with inflammation of unknown origin, the anemia of chronic disorders, HIV infection, acute infection or in chronic liver disorders, they may help distinguish these clinical problems from iron deficiency (Table 14). Indeed, sTfR levels may not be increased despite evidence of functional iron deficiency as evidenced by elevated zinc protoporphyrin. Furthermore, sTfR levels may decrease temporarily during acute inflammation (18).

**Table (2):** Differential diagnosis between iron deficiency anemia (IDA), anemia of chronic disorder (ACD) and the combination of ACD+ IDA (19)

<table>
<thead>
<tr>
<th></th>
<th>Hb</th>
<th>Serum iron</th>
<th>Ferritin</th>
<th>sTfR</th>
<th>sTfR/ferritin</th>
</tr>
</thead>
<tbody>
<tr>
<td>IDA</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>ACD</td>
<td>↓</td>
<td>↓</td>
<td>N–↑</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>ACD + IDA</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
</tr>
</tbody>
</table>

Blunted Epo production and suppression of erythropoiesis by cytokines may be the reasons for the absence of elevation of sTfR in the anemia of inflammation. On the other hand, patients with the anemia of chronic disorders may also have concomitant true iron deficiency and then show sTfR levels similarly elevated as in pure iron deficiency anemia (Table 14), even if these studies were not always based on a gold standard such as marrow iron determination. Values of sTfR decrease after adequate iron supplementation, independently of marrow iron stores, indicating that they are good indicators of functional iron deficiency (20).

Contrarily to serum ferritin, sTfR may thus prove to be a diagnostic test of iron deficiency in patients with inflammation. However, in some studies, sTfR levels could not distinguish accurately among patients with rheumatoid arthritis, inflammatory bowel disease or other inflammatory disorders, those with or without iron deficiency. In addition, several studies have observed higher sTfR levels in iron-replete rheumatoid arthritis patients compared to normal subjects, even if they remain lower than in iron deficiency anemia (15).
Similarly, sTfR levels were increased in a large group of patients with systemic lupus erythematosus but no information is available on their iron status. Levels were inversely related to Hb values, suggesting that they originated from stimulated erythropoiesis. Alternatively, this may indicate functional iron deficiency (21). Indeed, sTfR levels were elevated in cystic fibrosis patients with other signs of functional iron deficiency and were correlated with the degree of inflammation. Therefore, the cutoff point for the diagnosis of depleted iron stores in a patient with inflammation could be higher than the upper limit of normal values (21).

In addition, serum ferritin, with cutoff points ranging from 30 to 100 Ag/l, in some studies offered an equivalent or even better prediction of bone marrow iron content or of Hb response to iron supplementation. However, the combined use of sTfR and ferritin or the use of TfR/ferritin or TfR/log ferritin ratios may increase the efficacy of sTfR in identifying iron deficiency in patients with chronic inflammation. In particular, the log (sTfR/ferritin) ratio may prove superior to the sTfR/log ferritin index (21).

**Soluble TfR: a marker of iron status and/or erythropoiesis?**

Within the iron-replete range, sTfR correlates with Hb but not with markers of iron status such as transferrin saturation and ferritin. An inverse correlation between sTfR and ferritin may even represent an association between erythropoietic activity and iron utilization for erythropoiesis rather than an effect of iron stores on TfR expression (22).

Soluble TfR is therefore only a marker of erythropoiesis when iron stores are adequate and available and additionally becomes a marker of iron status only when tissue iron deficiency (with or without adequate iron stores) occurs. While sTfR has proved to be a reliable marker of tissue iron deficiency, the interpretation of an individual value may be tricky in a patient in whom both changes in erythropoietic activity and iron status may occur simultaneously. In unselected populations of patients with a variety of diagnoses, the specificity and positive predictive value of an elevated sTfR level for the diagnosis of iron deficiency is low because many patients have other reasons (predominantly increased erythropoiesis) for it. Combining sTfR and ferritin measurements may increase specificity but not sensitivity for the diagnosis of iron deficiency in such a population (22).

In patients with rheumatoid arthritis or other forms of anemia of chronic disorders, sTfR levels may well remain within the normal range even when iron stores are depleted because cytokines or other factors also suppress erythropoiesis directly or through inhibition of Epo production. On the other hand, levels may be elevated even when iron stores are adequate because marrow erythropoietic activity may be increased. Therefore, the relationship between iron status and sTfR levels in patients with inflammation depends on the severity of inflammation, the degree of anemia, the adequacy of Epo production and the effect of cytokines on marrow activity (22).

In other situations, as well, it may be difficult to distinguish the respective influence of erythropoiesis and iron deficiency on sTfR levels. Patients with chronic liver disease may have elevated sTfR levels when they experience either iron deficiency or stimulated erythropoiesis in response to hemolysis or hemorrhage. Renal failure patients have low sTfR levels as a result of erythroid hypoplasia and iron deficiency is then associated with a relative elevation of sTfR levels that nevertheless remain within the normal range (23).
Active malaria is associated with changes in erythropoietic activity brought about by hemolysis (which would tend to increase sTfR levels) as well as inflammation (which would rather decrease sTfR levels). The predominant effect was shown to be suppression of erythropoiesis by inflammation in active malaria, resulting in decreased sTfR levels and appropriate marrow response to mild anemia in asymptomatic infection, with elevated sTfR levels. Thus the diagnostic value of sTfR levels for iron deficiency may be impaired in individuals living in highly endemic areas for malaria (24).

The interpretation of sTfR values could be even more difficult in patients treated with rHuEpo, where erythropoietic response is often associated with functional iron deficiency. If sTfR was mostly a marker of functional iron deficiency induced by rHuEpo, it should increase more consistently in nonresponders (primary cause of failure is functional iron deficiency) than in responders: in fact the reverse is true (24). In addition, sTfR increments correlate well with later hematocrit increases, whereas levels are not different in patients with low or high ferritin and correction of iron deficiency with oral iron is associated with little changes. Furthermore, the elevation of sTfR in response to rHuEpo is larger in iron overloaded rats than in normal animals who develop functional iron deficiency. Therefore, sTfR remains predominantly a marker of erythroid response to rHuEpo (24).

Another situation where changes in iron status and erythropoiesis interact is pregnancy. Actually, sTfR levels are decreased in the first two trimesters, normalize in the first part of the 3rd trimester, and are slightly increased in late pregnancy and the early postpartum. These changes in erythropoietic activity are still well apparent after carefully excluding iron deficient women, parallel those in Epo production and reticulocytes, and thus explain known alterations in the red cell mass throughout pregnancy. On the other hand, some studies have found that depletion of iron stores also produced a moderate elevation of sTfR over levels observed in non-iron-deficient pregnancies, particularly at time of labor (25).

References


