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MONITORING OF ERYTHROPOIESIS BY THE SERUM TRANSFERRIN RECEPTOR AND ERYTHROPOIETIN[1]

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Key words: Transferrin, Serum transferring receptor, Erythropoietin, Erythropoiesis quantitation.

ABSTRACT

Virtually all cells have transferrin receptors (a transmembrane glycoprotein) on their surface, but in a normal adult, 80% of them are in the erythroid marrow. Some of them are lost into the circulation where they can be measured by immuno-assays. A direct and highly significant correlation exists between serum transferrin receptor level and erythron transferrin uptake in humans. The measurement of serum transferrin receptor has wide clinical applications for the quantitation of erythropoiesis. It can be used to study erythropoiesis in situations in which ferrokinetics is not acceptable, such as pregnancy. It is particularly useful for serial studies, i.e., for monitoring the recovery of erythropoiesis after stem cell transplantation or after treatment with erythropoietin. Combined with the determination of serum erythropoietin, both evaluated in relation to the degree of anemia, they provide a physiological approach to the diagnosis of anemia. Thus, the simultaneous determination of hematocrit, reticulocytes, serum transferrin receptor and serum erythropoietin has high discriminatory value in distinguishing between a defect in erythroid proliferation, maturation or red cell survival. It is also particularly useful for detecting the presence of multiple mechanisms of anemia in the same patient.

INTRODUCTION

Erythropoiesis is primarily dependant on erythroid progenitors, their stimulation by erythropoietin (Epo), and adequate supply of iron.
Epo production by the kidney is determined by the level of oxygen supply which mostly depends on the red cell mass. Epo stimulates red cell production by inducing the proliferation and differentiation of committed erythroid progenitors. In the presence of a normal marrow stem cell reserve, erythropoiesis will therefore increase in proportion to the degree of anemia. In the last ten years, new tools have been developed for the evaluation of erythropoiesis. This paper discusses the clinical relevance of serum transferrin receptor and Epo for the quantitation of erythropoiesis in man.

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IRON, TRANSFERRIN AND TISSUE TRANSFERRIN RECEPTOR

The internal iron cycle consists of iron uptake by immature erythroid cells which incorporate most of the iron taken up into hemoglobin, the subsequent circulation of the iron-containing red cell through the vascular system, the eventual processing of senescent or defective red cells by the reticuloendothelial cells (macrophages and Kupffer cells) and the return of iron to circulating transferrin. This cycle is driven by erythropoiesis.

Iron transport in the plasma is carried out by transferrin, a single chain glycoprotein of 80.000 Daltons. The transferrin gene is located on chromosome 3 and there is evidence that it derived from a gene duplication event. Transferrin synthesis takes place mostly in the hepatocyte and production is increased with iron store depletion and decreases with repletion. The plasma iron pool is heterogeneous, consisting of apo-, di-, and two forms of mono-ferric transferrin molecules. The two monoferric forms have identical iron-donating capacity both in terms of rate of donation and pattern to tissue distribution. However, there is a 3.5-fold preference of receptors for diferric compared with monoferric transferrin and removal of iron from plasma occurs preferentially from the pool of diferric molecules. Iron donation to cells is an all-or-none phenomenon in which both atoms of iron are removed simultaneously from diferric transferrin.

The endocytic pathway of transferrin and its receptor has been well characterized. At neutral pH, the transferrin receptor has very high affinity for diferric transferrin, intermediate affinity for monoferric transferrin, and very little for apotransferrin. On the other hand, it forms a very stable complex with apotransferrin at pH 5.0. After the binding of transferrin to its specific surface receptor, the complex is internalised in an endocytic vesicle or endosome. Due to the acidification of that organelle, iron is released from the iron-carrying transferrin. In contrast to most ligands which at that point dissociate from their receptor and enter a pathway leading to fusion with lysosomes and degradation, apotransferrin remains bound to its receptor because of the acidic pH. As with many receptor systems, the transferrin receptor is recycled to the cell membrane, carrying apotransferrin back to the cell surface where the neutral pH causes its release. The duration of the intracellular pathway is between 3 and 16 minutes.

Virtually all cells have transferrin receptors on their surface, but the largest numbers are in the erythron, placenta, and liver . In a normal adult about 80% of transferrin receptors are in the erythroid marrow, and that proportion even augments in situations of stimulated erythropoiesis. Receptors increase from low number on erythroid progenitors to a maximum of 800.000 per cell on the intermediate normoblasts, before declining to about 100.000 on reticulocytes and none on mature red cells. It is generally admitted that hepatocytes express

20.000-50.000 receptors. Receptor density on cells is related to the availability of iron as deprivation of iron results in prompt induction of transferrin receptor synthesis whereas excess iron suppresses receptor number (figure 1). This phenomenon is controlled at the transcription level.

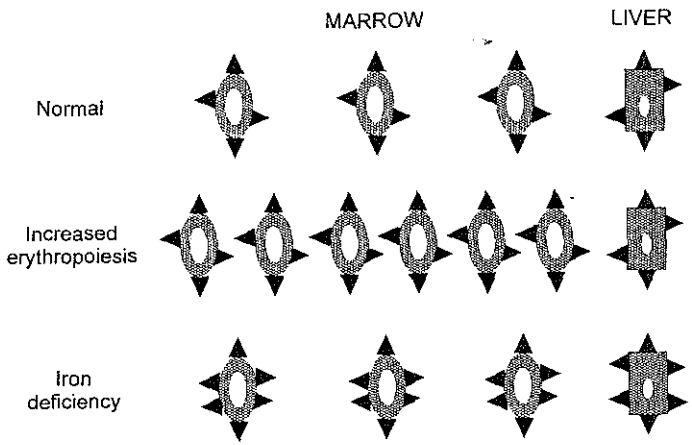

Figure 1: *Location and density of tissue TfR.*
In a normal adult, about 80% of TfR are in the erythroid marrow. With expansion of erythropoiesis, the number of TfR increases proportionally. Iron deficiency results in induction of TfR synthesis. TfR are represented as triangles at the surface of the cells.

CIRCULATING (SOLUBLE) TRANSFERRIN RECEPTOR

The soluble transferrin receptor (sTfR) has been identified in rat (1) as well as human serum (2). The soluble receptor is a truncated form of tissue receptor lacking its first 100 amino acids, probably derived by cleavage of the extracellular portion of the receptor (3). It circulates in serum in the form of a complex of transferrin and its receptor, possibly with two receptor monomers binding to one transferrin molecule, with a total molecular weight of about 250.000 (figure 2).
A number of quantitative assays, including radioimmunoassays and enzyme -linked immunoabsorbent assays using polyclonal or monoclonal antibodies, have now been set up to measure sTfR in biological fluids. In normal human subjects, receptor levels average 5.000 ± 1.100 ng/ml.

The level of erythropoietic activity has been found to be the most important determinant of sTfR levels (2, 4). Decreased sTfR levels are found in situations characterized by erythroid hypoplasia, such as hypertransfusion, chronic renal failure, severe aplastic anemia, or after intensive chemotherapy. Increased sTfR levels are seen in situations of hemolysis or stimulated ineffective erythropoiesis such as immune hemolytic anemia, hereditary spherocytosis, sickle cell anemia, thalassemia, megaloblastic anemia, or secondary polycythemia. Soluble

TfR levels range from a minimum of about 700ng/ml (which would represent the contribution of non-erythroid tissues to serum levels) when erythropoiesis is totally suppressed, to about 100.000 ng/ml in a severely anemic thalassemia patient. Increased expression of transferrin receptors has been documented on the surface of malignant tumor cells as compared to their normal counterparts. However, data on receptor levels in patients with a variety of myeloid, lymphoid and non-hematologic malignancies suggest that, with the exception of polycythemia vera, chronic lymphocytic leukaemia and high-grade non-Hodgkin's lymphoma, the possible contribution of neoplasic cells to plasma TfR levels is negligible.

The plasma iron turnover (PIT), in which the daily transport of iron through the plasma is calculated from the plasma iron and the radioiron disappearance curve after intravenous injection of a tracer dose has been extensively used as a quantitative measure of erythropoiesis (5, 6). This relationship is meaningful because about 80% of iron passing through the plasma is delivered to the erythroid marrow and iron uptake of other tissues remains relatively constant. Multicompartmental models of iron pathways have then been proposed to quantitate effective erythropoiesis, ineffective erythropoiesis, red cell lifespan, and non-erythroid iron turnover (7, 8). These studies are cumbersome to perform, usually two weeks in duration, and require repeated blood samplings and sophisticated computer calculations. The use of these models is also hampered by the fact that they are based on assumptions which may not all be valid, and that certain aspects of iron exchange are impossible to resolve. A simplified approach to the ferrokinetic evaluation of erythroid marrow activity has been proposed by *Cazzola*, and

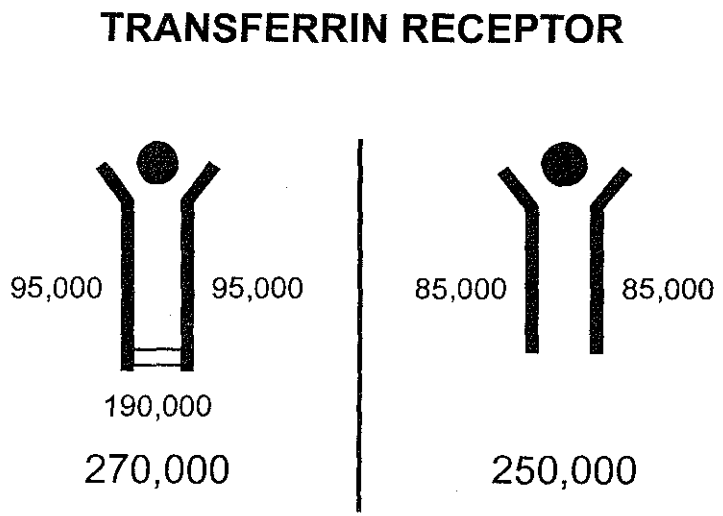

Figure 2: *Molecular forms of the TfR.*
The left panel represents cellular TfR composed of two monomers (MW 95.000 each) linked by two disulfide bridges, and capable of binding one transferrin molecule (Closed circle, MW 80.000), to give a total MW of 270.000. The right panel represents sTfR, a complex of Tf and two truncated monomers (MW 85.000 each), to give a total MW of 250.000.

the erythron transferrin uptake (ETU) is now established as the best available method for the quantitation of erythropoiesis (9, 10). The dependence of sTfR levels on the activity of the erythron is further demonstrated by the strong correlation observed between serum receptor and ferrokinetic measurements of erythropoiesis. This has been shown in rats and confirmed in humans in whom the relationship between mean erythron transferrin uptake (ETU) and mean sTfR level in subjects with a variety of diagnoses is very close to the line of proportionality (from 0,2 to 10 times normal) (11).

The second major determinant of circulating TfR is the body iron status. As compared to normal values, sTfR levels are about 25% lower in patients with idiopathic hemochromatosis and about 20% higher in non-anemic iron-deficient subjects. These variations are small compared to those determined by variations in erythropoiesis.

ERYTHROPOIETIN

Epo is a glycoprotein hormone consisting of a single chain of 165 amino acids and four carbohydrate chains containing a large amount of sialic acid, giving a molecular weight of 34.000 Daltons. Glycosylation is required for the survival of EPO in circulation, but not necessarily for its biological activity. The gene for erythropoietin is located on chromosome 7 and its transcription is activated by hypoxia.

Epo production takes place primarily in peritubular cells of the kidney that are probably endothelial cells or macrophages. Epo is also generated in the liver and in marrow macrophages, but this accounts for no more than 10-15% of total production and cannot compensate for loss of the renal source. Epo production is regulated through a feedback system between the bone marrow and the kidney which depends on a renal oxygen sensor, possibly a heme protein : Epo stimulates the bone marrow to produce red cells whose circulating mass determines the renal oxygen concentration.

Immunoassays have now replaced bioassays such as the polycythemic mouse assay or the mouse spleen cell assay for the measurements of serum Epo levels. Levels in normal individuals are in the range of 10 to 25 mU/ml.

In the laboratory diagnosis of anemia, however, serum Epo should not be quantitated in absolute terms. In fact, provided that the erythropoietin-generating apparatus in the kidney is efficient, levels increase exponentially as the hematocrit decreases. Consequently, serum Epo levels must be expressed in relation to the hematocrit. This can be done by determining the exponential regression of serum Epo versus hematocrit in reference subjects, and defining the 95% confidence limits. In clinical investigations, the adequacy of Epo response to anemia has been generally evaluated by comparing the regression curve of serum Epo versus hematocrit obtained in a group of patients with that of reference subjects (figure 3). When studying an individual patient, appropriateness of Epo response to anemia can be evaluated graphically as indicated in fig.6 or through the observed/predicted log(Epo) ratio (O/P ratio). The O/P ratio averaged 1.00 ± 0.11 in our reference subjects (95% confidence interval : 0.80- 1.19) : values lower then 0,80 indicate an inadequate Epo response to anemia.

The wide range of appropriate Epo levels for anemic patients suggest that factors other than tissue hypoxia influence serum Epo (12). In fact, at any given hematocrit level, serum Epo is higher in patients with low erythroid activity compared with patients with hyperplastic erythropoiesis. Thus the second major determinant of serum Epo is the red blood cell precursor mass (13). It remains to be determined whether the erythroid precursor mass acts directly by utilising circulating Epo or indirectly by influencing the rate of Epo production.

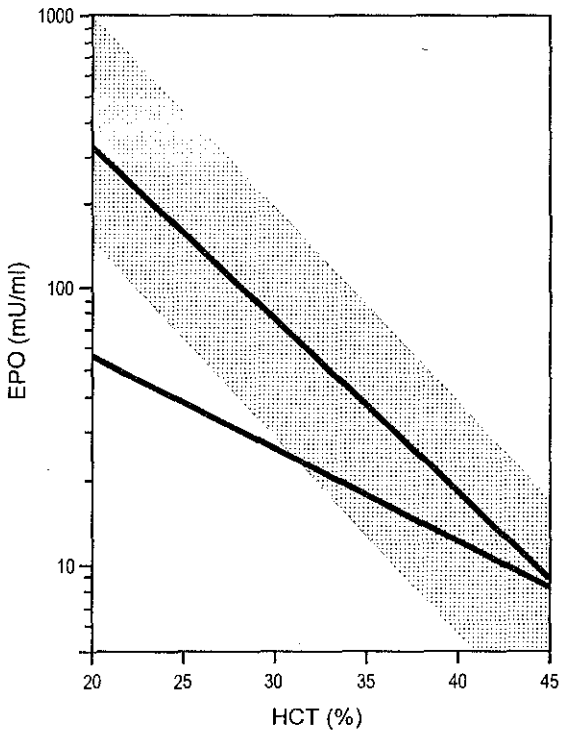

Figure 3: *Regression of Epo levels versus hematocrit in autologous (upper regression line) and allogenic (lower regression line) transplants. Control subjects are represented by their 95% confidence limits (shaded area). Reprinted from Blood (ref.17), with permission of the American Society of Hematology.*

ASSESSMENT OF ERYTHROPOIESIS BY SERUM TRANSFERRIN RECEPTOR AND ERYTHROPOIETIN DETERMINATIONS

Some applications are described along the following lines.

1. Measurement of erythropoiesis during pregnancy

Pregnancy causes a rise in plasma volume and red cell mass which reach respectively 150% and 120-125% of non-pregnant values near term. However, the total red cell mass first decreases in early pregnancy, before gradually returning to non-pregnant values by week 30 and further increasing in late pregnancy.

Using the serum TfR assay, it has been possible to show for the first time that important changes in the rate of erythropoiesis take place during pregnancy. In a large study, erythropoiesis

was shown to be depressed in early pregnancy, to normalize in the first part of the third trimester, and to increase slightly beyond normal values in late pregnancy, at delivery, and in the early postpartum (14). Almost 50% of women had receptor levels below the lower limit of normal around week 16, but virtually none after week 37. Although serum Epo levels increased slightly throughout pregnancy, when Epo levels were analysed in relation to the hematocrit, both individually by O/P ratio and collectively in regression analysis, it was shown that Epo production is impaired in early pregnancy, recovers in late pregnancy, and normalizes in the early postpartum (14, 15). When predicted Epo values were derived for each hematocrit and an O/P ratio of observed/predicted log (EPO) was calculated, there was a striking parallelism between Epo O/P ratio and TfR throughout pregnancy (figure 4). Therefore, impaired erythropoietin production is responsible for defective erythropoiesis in early pregnancy. Several physiologic adaptations to pregnancy may augment oxygen supply to the kidney sensor, thus depressing erythropoietin release in the first trimester.

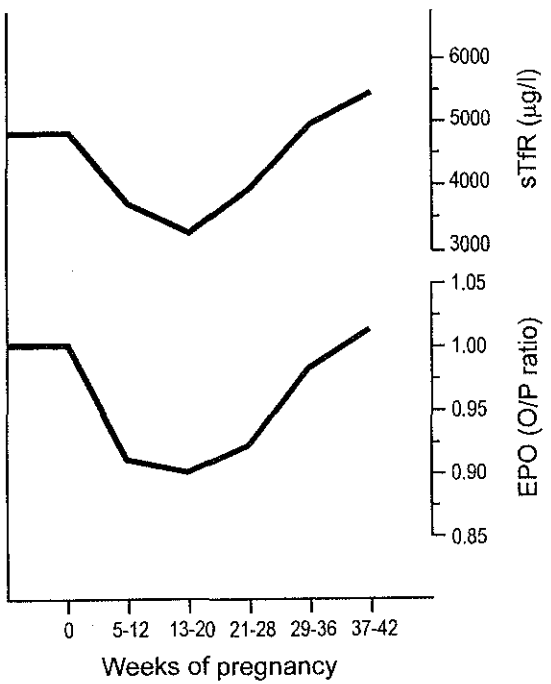

Figure 4: *Evolution of sTfR levels and Epo O/P ratios throughout pregnancy. Epo levels remain insufficient for the degree of anemia in the first part of pregnancy but return progressively to appropriate levels thereafter. These changes in Epo production are responsible for a relative erythropoietic failure and a correction thereafter.*

2. Recovery of erythropoiesis after stem cell transplantation

a) Bone marrow transplantation

The effect of various factors on the kinetics of neutrophil and platelet engraftment after allogenic (BMT) and autologous (ABMT) bone marrow transplantations has been extensively studied. Not much attention has been given erythrocyte recovery, partly because of lack of a reliable method to monitor erythropoiesis sequentially. Again, the sTfR assay provided the opportunity to investigate the recovery of erythropoiesis after marrow transplantation (16). Contrarily to platelet and neutrophil engraftment which are faster after BMT, erythrocyte recovery is significantly delayed after BMT as compared to ABMT. After an initial phase where it seems to follow the same course as in ABMT, erythropoiesis stays at insufficient intensity for months and reaches levels similar to those achieved after ABTM only after one year. Epo production in ABMT patients remains adequate throughout the post transplant course. After BMT, Epo levels rapidly become inappropriately low and stays so for prolonged periods of times (17). Therefore, the development of erythropoiesis after autologous transplantation is determined by the overall marrow proliferative capacity and Epo plays only a facilitating role. On the other hand, after allogenic transplantation, erythropoiesis depends on Epo levels which remains inadequate for prolonged periods of time (figure 3).

b) Peripheral blood progenitor cells (PBPC) transplantation

The more recent use of PBPC collected after chemotherapy and/or growth factor(s) administration has permitted a faster hematopoietic reconstitution than that seen with the transplantation of bone marrow cells. Since Epo production is appropriate in the autologous setting, this leads to rapid erythropoietic engraftment. Indeed, while TfR levels reach their nadir on day 7 both in PBCB and marrow transplants, sTfR returns to normal values by day 28 in PBPC transplants but not within 100 days in marrow transplants (18).

3. Erythropoiesis in chronic lymphocytic leukaemia and multiple myeloma

Chronic lymphocytic leukaemia (CLL) and multiple myeloma (MM) are hematologic malignancies where anemia develops with advancing stages (Binet, Durie & Salmon, staging systems). Anemia usually progresses in parallel with marrow infiltration, but several cytokines known to inhibit erythropoietin formation are produced by CLL B cells and myeloma cells.

In CLL, Epo levels are increased compared to normal individuals and this elevation appears adequate for the degree of anemia. The slope of the regression of Epo versus hematocrit is similar to that of a reference group. sTfR levels are also appropriately elevated for the degree of anemia and correlate with serum Epo. Advanced stage is not associated with reduction of Epo production but diminished erythropoietic activity can be observed in some patients. Therefore, anemia in CLL is not characterized by inadequate Epo production (19). Increased hemolysis and/or splenic sequestration certainly play a role.

Anemia is also a frequent complication of MM. It may occur even in the absence of massive marrow replacement by malignant plasmocytes and in the presence of normal leucocyte and platelet counts. When erythropoiesis is quantitated by serum transferrin receptor levels, it appears that the mechanism of anemia in MM is primarily defective red cell production. Erythropoiesis decreases and anemia worsens significantly with advancing clinical stage. 25% of the patients have inadequate Epo production and this proportion increases to 50% in stage 3. Inappropriate Epo production is seen in 60% of patients with renal impairment but is also observed in a number of patients with normal renal function. Erythropoiesis correlates

strongly with the adequacy of Epo production, particularly in advanced disease. Therefore, most myeloma patients have defective red cell production even in the absence of massive marrow infiltration and inappropriate Epo production contributes to their anemia (20).

4. Early prediction of response to recombinant human erythropoietin

In response to recombinant human erythropoietin (rHuEpo), the erythroblast compartment starts to respond within a few days but requires a period of 6 weeks to fully expand before reaching a steady state during which the hemoglobin continues to rise (21).

a) Anemia of chronic renal failure

rHuEpo has been shown to be effective in correcting the anemia of chronic renal failure, but the dose needed may be variable. In the first years of rHuEpo usage in renal failure, a study was conducted to assess the value of various laboratory parameters (baseline values and early changes) as predictors of response to rHuEpo. The best prediction by pre-treatment parameters only was obtained with baseline serum transferrin receptor (<or ≥ 3.500 ng/ml) and fibrinogen (< or ≥ 4 g/l) : 100% response rate when both parameters were low, versus only 29% when they were both high, and versus 67% when one was low and the other high. This illustrates the importance of functional iron deficiency (higher sTfR) and subclinical inflammation (higher fibrinogen) in impairing the response to rHuEpo. Evaluating the early erythropoietic response to rHuEpo before a significant change in hematocrit could be observed was also very useful. In hemodialysis patients, a sTfR increment of at least 20% after 2 weeks secured a response in virtually 100% of the patients. Thus, the response to rHuEpo could be predicted early by pre-treatment fibrinogen an sTfR, together with early changes of sTfR levels. Early recognition of a low probability of response in a given patient could help identify and correct specific causes of treatment failure to hasten clinical improvement and avoid prolonged ineffective use of an expensive medication (22). Nowadays, this implies the systemic use of IV iron supplementation to avoid functional iron deficiency. Being so, nearly all rHuEpo treated patients respond to therapy.

b) Anemia of cancer

Many studies have shown that rHuEpo therapy can ameliorate the anemia associated with cancer and chemotherapy, reduce the need for transfusions and improve the quality of life. However, a number of disease- or chemotherapy-related factors determines the probability of response. Several specific mechanisms of anemia, such as hemolysis, splenomegaly, bleeding, hemodilution or ineffective erythropoiesis can seriously interfere with response. Stem cell damage by previous therapy will impair response and marrow suppression by current intensive chemotherapy will also have a negative impact. Complications such as infections or nutritional deficiencies are important response-limiting factors. Because up to 40% of the patients will not respond to rHuEpo, it is of utmost importance to develop models that could help predict response to rHuEpo and thus select the most appropriate cancer patients for this therapy. The combined use of baseline serum Epo and the two 2-week sTfR increment proves to be very powerful, achieving an overall accuracy of 90%. However, early prediction of response based on sTfR elevation early in the course of treatment will not be valid in patients in whom rHuEpo essentially stimulates ineffective erythropoiesis, for instance patients with a myelodysplasic syndrome.

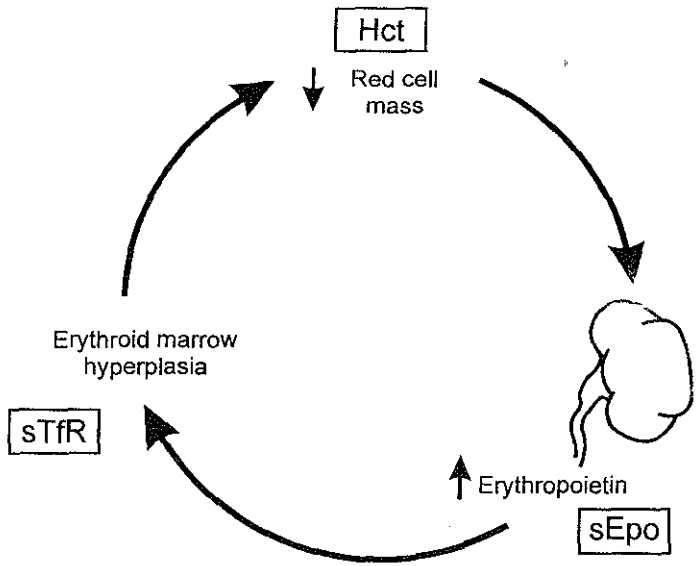

Figure 5: *A simple model of erythropoiesis.*
Anemia (measured by the Hct or Hb level) stimulates Epo production by the kidney (measured by serum EPO) which in turn stimulates erythropoiesis (measured by sTfR). The reticulocyte index discriminates between effective and ineffective erythropoiesis. The physiologic assessment of red blood cell disorders can be made in a single blood sample.

5. Functional classification of anemia based on measurements of serum transferrin receptor, erythropoietin and hematocrit

We evaluated the quantitative value of a simple model of erythropoiesis, based on the basic assumptions that the red blood cell mass determines erythropoietin production, which in turn stimulates erythropoietic activity (figure 5). Instead of only quantitating Epo and erythropoiesis in absolute terms, we also evaluated them in relation to the degree of anemia, and expressed the results as a ratio of observed values to values predicted from the regression equations between hematocrit one the one hand, and Epo and STfR on the other. The slope of the regression of sTfR versus hematocrit was very similar to the slope of the regression of Epo versus hematocrit (figure 6). We identified four major patterns of erythropoiesis, i.e., normal, hyperdestruction (with variants of hemolysis or ineffective erythropoiesis), intrinsic marrow hypoproliferation, and defective Epo production. The reticulocytes count and index was useful to separate hemolysis from ineffective erythropoiesis. Thus the pathophysiology

of anemia can be assessed by a simple measurement of hematocrit, retic index, Epo and sTfR levels, with Epo and sTfR being more informative when expressed in relation to the degree of anemia. The model is particularly useful for detecting the presence

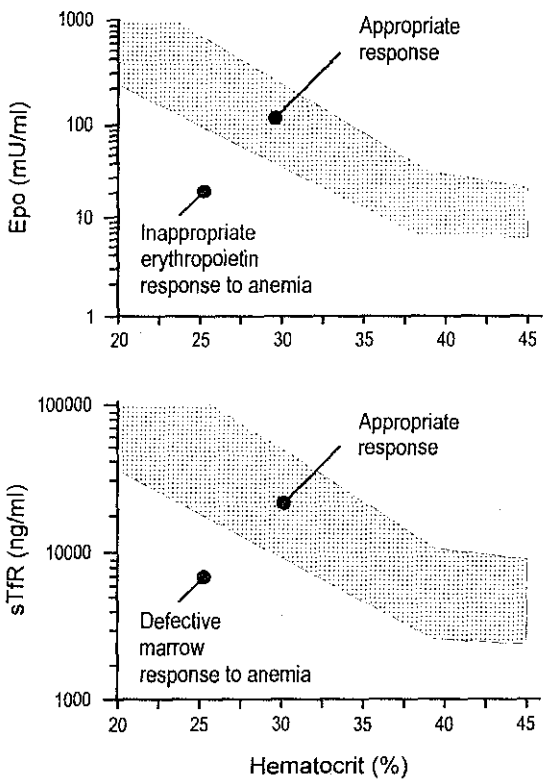

Figure 6: *Relationship between hematocrit and serum Epo (upper panel) or the sTfR (lower panel). In the presence of a normal Epo response and a normal marrow stem cell reserve, serum Epo and erythropoiesis both increase exponentially in proportion to the degree of anemia. Shaded areas are the 95% confidence limits. Values below this range indicate an inappropriate response to anemia.*

SOLUBLE TRANSFERRIN RECEPTOR AND IRON STATUS

sTfR is only a marker of erythropoiesis when iron stores are adequate and available but additionally becomes a marker of iron status when tissue iron deficiency occurs.

a) Iron deficiency anemia

sTfR level is considerably elevated in iron deficiency anemia and increases exponentially with the resulting enhanced erythropoietic stimulation.

b) Functional iron deficiency

Functional iron deficiency is an imbalance between iron needs in the erythropoietic marrow and iron supply, which depends on the level iron stores and its rate of mobilization. An elegant study has made a major contribution to the evaluation of sTfR as a marker of the iron status (24). When normal volunteers underwent graded phlebotomy, ferritin decreased progressively while sTfR did not change much during the phase of storage iron depletion. However, sTfR increased significantly when marrow functional iron deficiency and anemia developed. Therefore, the iron status may be fully assessed by using serum ferritin as a measure of iron stores (storage iron depletion), sTfR as a measure of functional tissue iron deficiency (iron deficient erythropoiesis), and hemoglobin as a measure of advanced iron deficiency (iron deficiency anemia).

c) Inflammation

sTfR remains normal in the anemia of chronic disorders. This may help distinguish this clinical problem from iron deficiency. Blunted Epo production and suppression of erythropoiesis by cytokines are the main reasons for the absence of elevation of sTfR in the anemia of inflammation.

d) Concomitant iron deficiency and inflammation

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. Contrarily to serum ferritin, sTfR may thus prove to be a diagnostic test of iron deficiency in patients with inflammation.

CONCLUSION

The direct relationship to the number of erythroid precursors and the simple methodology make sTfR assay the method of choice for evaluation of erythroid marrow activity in a clinical setting (iron kinetics in a test tube). The measurement of sTfR has wide clinical applications for quantitation of erythropoiesis in humans and can replace iron kinetics to the same extent that measurement of serum ferritin has replaced bone marrow aspiration for quantitation of iron stores. Combined with the measurement of sEpo and reticulocytes count, all evaluated in relation to the degree of anemia, they provide a functional classification of red cell disorders : defect in proliferation (hypoproliferative anemia), maturation (ineffective erythropoiesis), or red cell survival (peripheral hemolysis). They are a simple and non invasive method for the diagnosis of blunted erythropoietin production or inadequate erythroid marrow response to anemia. sTfR is particularly useful for serial studies and for monitoring erythropoiesis during rHuEpo treatment. sTfR is only a marker of erythropoiesis when iron stores are present and can be readily mobilized. In addition, it is a marker of iron status when tissue iron deficiency occurs.

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REFERENCES

1. BEGUIN Y, HUEBERS HA, JOSEPHON B, FINCH CA. *Transferrin receptors in rat plasma.* Proc.Natl.Acad.Sci.USA, 1988, 85: 637-40.
2. HUEBERS HA, BEGUIN Y, POOTRAKUL P, EINSPAHR D, FINCH CA. *Intact transferrin receptors in human plasma and their relation to erythropoiesis.* Blood, 1990, 75: 102-7.
3. SHIH YJ, BAYNES RD, HUDSON BG, FLOWERS CH, SKIKNE BS, COOK JD. *Serum transferrin receptor is a truncated form of tissue receptor.* J.Biol.Chem, 1990, 265: 19077-81.
4. BEGUIN Y, LOO M, R'ZIK S, SAUTOIS B, FILLET G. *Significance of soluble transferrin receptor.* In: Molecular Biology of Hematopoiesis, 1994, vol. 3 Abraham NG, Shadduk RK, Levene A, Takaku F, Eds Intereeps, Andover. Pp.451-64.
5. HUFF RL, HENNESSY TG, AUSTIN RE, GARCIA JF, ROBERTS BM, LAWRENCE JH. *Plasma and red cell iron turnover in normal subjects and in patients having various hematopoietic disorders.* J.Clin.Invest., 1950, 29: 1041-52.
6. DEUBELBEIS KA, COOK JD, HARKER LA, FINCH CA. *The ferrokinetic measurement of marrow cellularity.* In: Platelets kinetics. Radioisotopic, cytological, mathematical and clinical aspects, edited by Paulus JM. Amsterdam: North-Holland Publishing Co., pp. 161-6, 1971.
7. NAJEAN Y, DRESCH C, ARDAILLOU N, BERNARD J. *Iron metabolism study of different kinetic models in normal conditions.* Am.J.Physiol., 1967, 213: 533-46.
8. COOK JD, MARSAGLIA G, ESCHBACH JW, FUNK DD, FINCH CA. *Ferrokinetics : a biologic model for plasma iron exchange in man.* J.Clin.Invest., 1970, 49: 197-205.
9. CAZZOLA M, HUEBERS HA, SAYERS MH, MACPHAIL AP, ENG M, FINCH CA. *Transferrin saturation, plasma iron turnover, and transferrin uptake in normal humans.* Blood., 1985, 66: 935-9.
10. CAZZOLA M, POOTRAKUL P, HUEBERS HA, ENG M, ESCHBACH J, FINCH CA. *Erythroid marrow function in anemic patients.* Blood, 1987, 69: 296-301.
11. BEGUIN Y. *The soluble transferrin receptor : biological aspects and clinical usefulness as a quantitative measure of erythropoiesis.* Haematologica, 1992, 77: 1-10.
12. NAETS JP, WITTEK M. *Effect of erythroid hypoplasia on utilization of erythropoietin.* Nature, 1965, 206: 726-7.
13. CAZZOLA M, GUARNONE R, CERANI P, CENTENARA E, ROVATI A, BEGUIN Y. *Red blood cell precursor mass as an independent determinant of serum erythropoietin level.* Blood, 1998, 91: 2139-45.
14. BEGUIN Y, LIPSCEI G, THOUMSIN H, FILLET G. *Inappropriate erythropoietin production is responsible for defective erythropoiesis in early pregnancy.* Blood, 1991, 78: 89-93.
15. BEGUIN Y, LIPSCEI G, ORIS R, THOUMSIN H, FILLET G. *Serum immunoreactive erythropoietin during pregnancy and in the early postpartum.* Brit. J. Haematol., 1990, 76: 545-9.
16. BEGUIN Y, ORIS R, FILLET G. *Dynamics of erythropoietic recovery following bone marrow transplantation : role of marrow proliferative capacity and erythropoietin production in autologous versus allogenic transplants.* Bone marrow transplant, 1993, 11: 285-92.

17. BEGUIN Y, CLEMONS GK, ORIS R, FILLET G. *Circulating erythropoietin levels after bone marrow transplantation : inappropriate response to anemia in allogenic transplants.* Blood, 1991, 77: 868-73.
18. BEGUIN Y, BARON F, FILLET G. *Influence of marrow erythropoietic activity on serum erythropoietin levels after autologous hematopoietic stem cell transplantation.* Haematologica, 1998, 83: 1076-81.
19. BEGUIN Y, LAMPERTZ S, BRON D, FILLET G. *Serum erythropoietin in chronic lymphoid leukaemia.* Br.J.Haematol., 1996, 93: 154-6.
20. BEGUIN Y, YERNA M, LOO M, WEBER M, FILLET G. *Erythropoiesis in multiple myeloma: defective red cell production due to inappropriate erythropoietin production.* Brit.J.Haematol., 1992, 82: 648-53.
21. BEGUIN Y, LOO M, R'ZIK S, LEJEUNE F, SAUTOIS B, RORIVE G, FILLET G. *Quantitative assessment of erythropoiesis in hemodialysis patients demonstrates gradual expansion of erythroblasts during constant treatment with recombinant human erythropoietin.* Brit. J. Haematol., 1995, 89: 17-23.
22. BEGUIN Y, LOO M, R'ZIK S, SAUTOIS B, LEJEUNE F, RORIVE G, FILLET G. *Early prediction of response to recombinant human erythropoietin in patients with the anemia of chronic renal failure by serum transferrin receptor and fibrinogen.* Blood, 1993, 82: 2010-16.
23. BEGUIN Y, CLEMONS GK, POOTRAKUL P, FILLET G. *Quantitative assessment of erythropoiesis and functional classification of anemia based on measurements of serum transferrin receptor and erythropoietin.* Blood, 1993, 81: 1067-76.
24. SKIKNE BS, FLOWERS CH, COOK JD. *Serum transferrin receptor: a quantitative measure of tissue iron deficiency.* Blood, 1990, 75: 1870-6.