Clinical Study

Prevalence of Anaemia and Evaluation of Transferrin Receptor (sTfR) in the Diagnosis of Iron Deficiency in the Hospitalized Elderly Patients: Anaemia Clinical Studies in Chile

Mauricio López-Sierra,1 Susana Calderón,1 Jorge Gómez,2 and Lilian Pilleux1

1 Hematology Unit, Institute of Medicine, Faculty of Medicine, Universidad Austral de Chile, Buena 1003 CP 5090000 Valdivia, Chile
2 Institute of Public Health, Faculty of Medicine, Universidad Austral de Chile, Valdivia, Chile

Correspondence should be addressed to Mauricio López-Sierra, mlopezs@uach.cl

Received 24 October 2011; Revised 7 February 2012; Accepted 29 February 2012

Academic Editor: Lodovico Balducci

Copyright © 2012 Mauricio López-Sierra et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Iron constitutes the most prevalent nutritional deficiency worldwide. In Chile, anaemia epidemiological data is scarce, evaluating mainly children and women. Our objective was to determine prevalence of anaemia in an inpatient elderly population (≥60 years) and assess the usefulness of sTfR levels analyzed by other authors as a good predictor in the differential diagnosis of iron deficiency anaemia and anaemia of chronic disease.

Method. We studied medical patients admitted at Hospital of Valdivia (HV), Chile, in a 2month period. World Health Organization criteria were used for anaemia.

Results. 391 patients were hospitalized, average age 62.5 years, 247 elderly and 99 of which had anaemia. Anaemia was normocytic in 88.8%, and we observed: low serum iron in 46.3%, low ferritin 10.1%, high TIBC 2%, low % transferrin saturation (Tsat) 40%, and high sTfR 25%.

Conclusions. As a first figure known in Chile, the prevalence of anaemia in the elderly inpatient was 40.1%. Our findings encourage us to promote the implementation of sTfR determination in the clinical setting to analyze the state of erythropoiesis in patients with chronic diseases which commonly occurs in elderly.

1. Introduction

Iron (Fe) is an essential metal ion for living beings; although it is the fourth most abundant mineral in the earth’s crust, it is the most prevalent nutritional deficiency worldwide [1]. It participates in a variety of vital physiological processes such as oxygen transportation, energy production in the brain by cytochrome oxidase, enzymatic cofactor in the synthesis of neurotransmitters and myelin [2, 3]. The main consequence of iron deficiency is the generation of anaemia which allows us to estimate its prevalence in a given population indirectly by red blood cell counting. However, this approach has the limitation of including other aetiologies. The worldwide prevalence of iron deficiency is approximately 30%, resulting in close to 2 billion people with anaemia of this cause. In developing countries, the prevalence of anaemia among pregnant women and children under two years exceeds 50% [4, 5]. In Chile, the epidemiological anaemia data is scarce and sectored, with values ranging from 5.1% in women [6] up to 36% in infants of low socioeconomic status [7].

The impact of iron deficiency occurs not only in the hematopoietic system and is more evident in the early stages of life affecting preschool children who suffer from behavioral and affective disorders [8], increased infection susceptibility, and pregnant women having increased risk of preterm delivery, low birth weight, and death in the newborn (NB). Children, especially premature NB children from mothers with iron deficiency, adolescent girls, and women of childbearing age [9, 10] represent the most vulnerable population for this deficiency because of their increased demand and/or physiological loss of Fe.

There is no epidemiological data available of iron deficiency in the elderly, but they are expected to have a
higher prevalence of anaemia than in the general population, since longevity is associated with a variety of physiological dysfunctions, chronic and inflammatory diseases, and occasionally inadequate diet that lower reserves and availability of Fe. Clinical manifestations of anaemia in the elderly add to changes in sensory organs, increasing the risk of falls, with a decline in mobility and loss of autonomy [11] that results in an increase in health expenses.

When facing a patient with iron deficiency anaemia (IDA), the hematimetric and ferrokinetic classical standards can be altered by concomitant anaemia of chronic disease (ACD) secondary to infectious, neoplastic, or inflammatory diseases [12–15]. ACD is the consequence of the production of pro-inflammatory cytokines (IL1, 6, TNFα) [15] and some anti-inflammatory cytokines (IL-10) which induce the reticuloendothelial system to store Fe limiting its availability for erythropoiesis, decrease the half-life of erythrocytes, inhibit the production of erythropoietin (Epo) and decrease the sensitivity of erythroid precursors to Epo [15, 16]. Thus, ACD by itself results in hypoferremia and hyperferritinemia thereby complicating etiological diagnosis of patients with simultaneous IDA. Moreover, normal physiological levels of serum iron are difficult to establish in a population due to its circadian rhythm [18], technical limitations of the method, and frequent indication of ferrous salts [19].

In Fe deficiency, the decreased serum iron concentration leads to an increase in total capacity of iron binding (TIBC) and a decreased saturation of the iron transporter transferrin (Tsat). Ferritin (Ferr) and transferrin (Tf) have the disadvantage of being acute phase reactants with limited value in the differential diagnosis of ACD from IDA [20].

The above considerations justify efforts to design a highly sensitive and specific test to detect iron deficiency, ideally before the development of anaemia [21, 22]. Staining of the iron deposits in bone marrow (BM) remains as the gold standard before the development of anaemia [21, 22]. When facing a patient with iron deficiency anaemia (IDA), the hematimetric and ferrokinetic classical standards can be altered by concomitant anaemia of chronic disease (ACD) secondary to infectious, neoplastic, or inflammatory diseases [12–15]. ACD is the consequence of the production of pro-inflammatory cytokines (IL1, 6, TNFα) [15] and some anti-inflammatory cytokines (IL-10) which induce the reticuloendothelial system to store Fe limiting its availability for erythropoiesis, decrease the half-life of erythrocytes, inhibit the production of erythropoietin (Epo) and decrease the sensitivity of erythroid precursors to Epo [16, 17]. Thus, ACD by itself results in hypoferremia and hyperferritinemia thereby complicating etiological diagnosis of patients with simultaneous IDA. Moreover, normal physiological levels of serum iron are difficult to establish in a population due to its circadian rhythm [18], technical limitations of the method, and frequent indication of ferrous salts [19].

In Fe deficiency, the decreased serum iron concentration leads to an increase in total capacity of iron binding (TIBC) and a decreased saturation of the iron transporter transferrin (Tsat). Ferritin (Ferr) and transferrin (Tf) have the disadvantage of being acute phase reactants with limited value in the differential diagnosis of ACD from IDA [20].

The above considerations justify efforts to design a highly sensitive and specific test to detect iron deficiency, ideally before the development of anaemia [21, 22]. Staining of the iron deposits in bone marrow (BM) remains as the gold standard, but it is an invasive technique. We assessed the presence of anaemia through the first in-patient complete blood count (CBC) using the World Health Organization definition: Hemoglobin (Hb) <13 g/dL for males and 12 g/dL for women. Anaemia was considered microcytic when MCV was ≤80 fl, macrocytic ≥100 fl, and normocytic 81–99 fl. The severity of anaemia was considered to be severe: (Hb < 7 g/dL), moderate (Hb 7–9 g/dL) and mild (Hb 9–11 g/dL). Patients with anaemia were further for analyzed serum iron, serum ferritin, TIBC, Tsat and sTfR quantification. The techniques used in the study and their normal values according to the manufacturer are shown in Table 1.

The ratio sTfR/logFerr was calculated in patients with ferritin <30 ng/mL considering the value <1 as compatible with ACD, >2 as ACD associated with another etiology using the flowchart of Weiss and Goodnough [23]. Statistical analysis was expressed as mean ± SD or range of distribution if not distributed normally. Statistical calculations were performed with Epidat 3.1.

3. Results

During the study period, 391 patients were hospitalized, out of which 247 were over 60 years. Details of their socio-demographic variables are shown in Table 2. It was not possible to survey 44 patients because of their clinical features (confusion, dementia, etc.).

The patients hospitalized (n = 391) had an average hospitalization time of 6.3 days, with an average age of 62.5 years (15–95 years) of which a 63.15% were elderly. The CBC assessment revealed 99 elderly patients with anaemia according to the WHO criteria. This allowed us to calculate a prevalence of anaemia of 40.1% in the elderly inpatient population. In this population, the mean age was 73.2 years (60–90); the distribution by sex was 59.6% female and 40.4% male. The first three causes of hospitalization were in order of frequency: acute lung infection 12.5%, heart failure 10.2% and acute coronary syndrome with a 9.9%. Analysis of the haematological parameters revealed an anaemia distribution according to morphology of 88.8% normocytic, 9.1% microcytic and 2% macrocytic; and according to severity: severe 3% (Hb < 7 g/dL), moderate 25% (Hb 7–9 g/dL), mild 39% (Hb 9–11 g/dL), and of lower intensity 32% (Hb 11 to 12.9 g/dL). Ferrokinetic analysis revealed (see Figure 1) that serum iron was low in 50.5%, normal in 47.5%, and high in 2%; ferritin was low in 10.1%, normal in 52.5%, and high in 37.4%; TIBC was low in 73.5%, normal in 23.5%, and
Table 1: Techniques for ferrokinetics and haematological parameters and normal values according to manufacturer and iron deficiency criteria used in the study.

| Parameter                        | Method                                                                 | Manufacturer's normal value                  | Iron deficiency criteria                      |
|----------------------------------|------------------------------------------------------------------------|----------------------------------------------|-----------------------------------------------|
| Serum iron                       | Iron liquicolor photometric colorimetric test for iron with lipid clearing | Men 59–148 ug/dL; Women 37–145 ug/dL         | Men < 59 ug/dL; Women < 37 ug/dL              |
| Total iron-binding capacity (TIBC) | TIBC test, iron saturation and aluminum oxide absorption method, human | 250–370 ug/dL                                | >370 ug/dL                                    |
| Ferritin (Ferr)                  | Electrochemiluminescence immunoassay. Roche, Elecys 2010/modular analytics | 30–300 ng/mL                                 | <30 ng/mL                                     |
| % Transferrin saturation (%Tsat) | TIBC test/iron liquicolor, human                                       | 20%–45% ref                                 | <20%                                          |
| Soluble transferrin receptor (sTfR) | Enzyme-linked quantikine IVD human sTfR immunoassay                  | 8.7–28.1 nmol/L                              | >28.1 nmol/L                                  |
| Ratio sTfR/log Ferritin (sTfR/log Ferr) |                                                                               | 1-2                                          | >2                                            |
| MCV                              | SYSMEX. XE-alphaN automated hematology system roche,                   | 80–99 fl                                     | ≤80 fl                                        |
| CHCM                             | SYSMEX. XE-alphaN, automated hematology system roche,                   | 32–36 ug/dL                                  | ≤32 ug/dL                                     |

Table 2: Main demographic variables of hospitalized patients, elderly and those elderly with anaemia.

|                                | Total patients admitted | Elderly patients | Elderly patients with anaemia |
|--------------------------------|-------------------------|------------------|------------------------------|
| Number of cases                | 391                     | 247              | 99                           |
| Mean Age (years)               | 62.5                    | 73               | 73.2                         |
| Residence                      |                          |                  |                              |
| Urban                          | 280                     | 180              | 65                           |
| Rural                          | 95                      | 60               | 27                           |
| Not available                  | 16                      | 7                | 6                            |
| Literacy                       |                          |                  |                              |
| Yes                            | 288                     | 161              | 46                           |
| No                             | 47                      | 41               | 20                           |
| Not available                  | 56                      | 45               | 33                           |
| Gender (%)                     |                          |                  |                              |
| Male                           | 192                     | 118              | 40                           |
| Female                         | 199                     | 129              | 59                           |

Figure 1: Schematic representation of the number of patients in each category according to their ferrokinetic tests results.

High iron (n = 49) Low ferritin (n = 10)
Low sTfR (n = 24) Tsat (%) ( n = 39)

According to sTfR levels, patients were grouped into low 1%, normal 74%, and high 25%. Using the ferritin and sTfR/log Ferr values according to the algorithm suggested by Weiss [19], we concluded that there were 67% of ACD, 13.5% of IDA, 9.3% a combination of both, while 1.2% could not be categorized. The statistical analysis comparing the sensitivity and specificity of the sTfR/LogFerr ratio versus the other ferrokinetic parameters (Serum iron, TIBC, Tsat, ferritin, sTfR) was obtained from receiver operating characteristic (ROC) curves (Figure 2). The following areas under the curve were obtained for serum iron 0.2487, Tsat 0.1938 and ferritin 0.1061. sTfR and TIBC areas under curve was better: 0.9609; 0.7538, respectively. But when comparing the areas under the curve of sTfR and TIBC, any of them is a better diagnostic support if we do not have other test (e.g.: Perl’s stain), since both have asymptotic significance less than 0.05. However, the confidence interval for TIBC test was significantly lower than sTfR, which was a statistically significant difference.
4 Anemia

Sensibility

1- specificity

MCV

Serum iron

TIBC sat (%)

Ferritin

sTfR

0.9

0.8

0.7

0.6

0.5

0.4

0.3

0.2

0.1

0

1

0.90.80.70.60.50.40.30.20.10

1

0

0.1

0.2

0.3

0.4

0.5

0.6

0.7

0.8

0.9

1

Figure 2: Receiver operating characteristic (ROC) curves for Serum iron, TIBC, Ferritin, % Tsat and sTfR for elderly patients with anemia. sTfR/log Ferr ratio was considered the gold standard.

between the two areas under described under the ROC curves ($\chi^2$ test of homogeneity $P = 0.0214$).

4. Conclusions

From an epidemiological point of view, the prevalence of anaemia was 40.1% for elderly hospitalized patients which is higher than that reported in other age groups in Chile and was unknown until now [6, 31, 32].

Our study confirms that sTfR quantification is a valid method to analyse the erythropoiesis in several diseases. To evaluate the clinical usefulness of sTfR in elderly patients for determining their iron deficiency status, receiver operating characteristic (ROC) curves were used, and the maximum discrimination cut-off point was calculated (see Figure 2). The sTfR was highly superior (sTfR area under ROC curve = 0.9609) to discriminate IDA from ACD in an adult patient population as compared to the classical ferrokinetics and haematological parameters. Ferrokinetic studies in the analyzed population were not useful for evaluating iron reserves and even were misleading in the diagnosis. A low ferritin concentration had been described as a good parameter for diagnosing iron deficiency anaemia [33] however, it only managed to predict 10% of patients with anaemia of this type in our study. The low Tsat was consistent with iron deficiency anaemia in 25%, but did not discriminate with ACD. This is consistent with the fact that transferrin, similar to ferritin, is an acute phase reactant which is elevated in inflammatory disorders [34], still both are widely used [19] in clinical practice due to the simpler technique and low cost. Furthermore, low levels of transferrin or TIBC may be due to increased degradation rather than decreased synthesis, as a result of increased protein catabolism secondary to catabolic or anabiotic proinflammatory cytokines [35, 36]. Our study confirms that the analysis of different individual ferrokinetic parameters is of little use in patients with concomitant systemic diseases, which are especially prevalent in the elderly; see ROC curves and areas.

In elderly adults requiring hospitalization, ACD was the primary cause of anaemia constituting over 75% of cases, while only 10% were due to iron deficiency. Folic acid and/or vitamin B12 deficiencies were not analyzed because it was beyond the scope of this study. Finally, the method of excellence to evaluate iron deposits remains bone marrow aspirate analysis with Perls stain constituting in some cases the only technique for diagnosing iron deficiency, however, this test cannot be performed routinely because it is invasive, expensive and slow [37, 38]. Our work shows that quantification of sTfR is more sensitive and specific for discriminating iron deficiency anaemia from anaemia associated with inflammatory events, consistent with work from others authors [21, 23, 39, 40], and will be a useful diagnostic tool for the future in our elderly patients. It is also necessary to note that most of the study population was admitted to the hospital primarily for cardiac diseases (29.22%), respiratory diseases (13.14%), and renal failure (6.17%). The latter is directly related to the generation of anemia by a low secretion of Epo, which must be considered when making the sTfR test if a patient with impaired renal function.

This sTfR/logFerr ratio gives us an inverse linear relationship of the iron stores state and has the advantage of combining the increase of one parameter (sTfR) and the decline of another (ferritin) [21, 41]. Our findings encourage us to promote the implementation of this determination (sTfR) in the clinical setting as was previously proposed [24, 26, 42], and high interest for the elderly adults. It is important to note that this method still requires international standardization in order to define normal ranges, which at the present time limits its routine clinical application [43].

Authors’ Contribution

M. L-S. and L. P. responsible for the study design, performed the analysis, interpreted data, and wrote the manuscript; S. C. and L. P. obtained the patients data. J. G. performed the statistical analysis.

Conflict of Interests

The authors declare no competing financial interests.

Acknowledgments

This work has been supported by a Grant from the Colaboración Docente Asistencial Servicio de Salud Valdivia and Universidad Austral de Chile (COLDAS2007). Informed consent form was explained and applied.
Anemia

References

[1] E. DeMaeyer and M. Adiels-Tegman, "The prevalence of anemia in the world," World Health Statistics Quarterly, vol. 38, no. 3, pp. 302–316, 1985.

[2] B. T. Felt, J. L. Beard, T. Schallert et al., “Persistent neurochemical and behavioral abnormalities in adulthood despite early iron supplementation for perinatal iron deficiency anemia in rats,” Behavioural Brain Research, vol. 171, no. 2, pp. 261–270, 2006.

[3] A. H. van Vliet, J. M. Ketley, S. F. Park, and C. W. Penn, “The role of iron in Campylobacter gene regulation, metabolism and oxidative stress defense,” FEMS Microbiology Reviews, vol. 26, no. 2, pp. 173–186, 2002.

[4] E. Pollitt, Malnutrition and Infection in the Classroom, UNESCO, Paris, France, 1990.

[5] World Health Organization, Report of WHO/UNICEF/UNU Consultation on Indicators and Strategies for Iron Deficiency and Anemia Programmer. Draft IDA REP.01 on Iron Deficiency Anemia. Consultation on Indicators and Strategies for Iron Deficiency

[6] Ministerio de Salud and Gobierno de Chile, “Encuesta Nacional de Salud,” 2003, http://www.minsal.cl/.

[7] C. Artigas, L. Gonzalez, C. Hidalgo, M. Vera, and S. Munoz, “Prevalence of iron deficiency anemia in unweaned infants of Temuco: Chile,” Revista Chilena de Ciencias Médico-Biológicas, vol. 7, no. 2, pp. 61–66, 1997.

[8] B. Lozoff, F. Corapci, M. J. Burden et al., “Preschool-aged children with iron deficiency anemia show altered affect and behavior,” Journal of Nutrition, vol. 137, no. 3, pp. 683–689, 2007.

[9] J. M. Bourre, “Effects of nutrients (in food) on the structure and function of the nervous system: update on dietary requirements for brain. Part 1: micronutrients,” Journal of Nutrition, Health and Aging, vol. 10, no. 5, pp. 377–385, 2006.

[10] J. M. Bourre, “Effects of nutrients (in food) on the structure and function of the nervous system: update on dietary requirements for brain. Part 2: macronutrients,” Journal of Nutrition, Health and Aging, vol. 10, no. 5, pp. 386–399, 2006.

[11] "Anemias secondary to chronic disease and systemic disorder's," in Wintrobe's Clinical Hematology, chapter 47, Lippincott Williams & Wilkins, Baltimore, Md, USA, 2nd edition, 2004.

[12] E. R. Burns, S. N. Goldberg, C. Lawrence, and B. Wenz, “Clinical utility of serum tests for iron deficiency in hospitalized patients,” American Journal of Clinical Pathology, vol. 93, no. 2, pp. 240–245, 1990.

[13] D. A. Lipschitz, "The anemia of chronic disease," Journal of the American Geriatrics Society, vol. 38, no. 11, pp. 1258–1264, 1990.

[14] J. M. Cash and D. A. Sears, "The anemia of chronic disease: spectrum of associated diseases in a series of unselected hospitalized patients," American Journal of Medicine, vol. 87, no. 6, pp. 638–644, 1989.

[15] B. B. Aggarwal, S. Shishodia, S. K. Sandur, M. K. Pandey, and G. Sethi, "Inflammation and cancer: how hot is the link?" Biochemical Pharmacology, vol. 72, no. 11, pp. 1605–1621, 2006.

[16] D. Yoon, Y. D. Pastore, V. Divoky et al., "Hypoxia-inducible factor-1 deficiency results in dysregulated erythropoiesis signaling and iron homeostasis in mouse development," The Journal of Biological Chemistry, vol. 281, no. 35, pp. 25703–25711, 2006.

[17] G. Noe, J. Augustin, S. Hausdorf, I. N. Rich, and B. Kubanek, "Serum erythropoietin and transferrin receptor levels in patients with rheumatoid arthritis," Clinical and Experimental Rheumatology, vol. 13, no. 4, pp. 445–451, 1995.

[18] A. S. Zhang and C. A. Enns, "Iron homeostasis: recently identified proteins provide insight into novel control mechanisms," The Journal of Biological Chemistry, vol. 284, no. 2, pp. 711–715, 2009.

[19] G. Weiss, "Pathogenesis and treatment of anemia of chronic disease," Blood Reviews, vol. 16, no. 2, pp. 87–96, 2002.

[20] H. Tilg, H. Ulmer, A. Kaser, and G. Weiss, "Role of IL-10 for induction of anemia during inflammation," Journal of Immunology, vol. 169, no. 4, pp. 2204–2209, 2002.

[21] K. Punnonen, K. Irjala, and A. Rajamäki, "Serum transferrin receptor and its ratio to serum ferritin in the diagnosis of iron deficiency," Blood, vol. 89, no. 3, pp. 1052–1057, 1997.

[22] J. W. Choi, "Sensitivity, specificity, and predictive value of serum soluble transferrin receptor at different stages of iron deficiency," Annals of Clinical and Laboratory Science, vol. 35, no. 4, pp. 435–439, 2005.

[23] G. Weiss and L. T. Goodnough, "Anemia of chronic disease," The New England Journal of Medicine, vol. 352, no. 10, pp. 1011–1023, 2005.

[24] M. Markovic, N. Majkic-Singh, and V. Subota, "Usefulness of soluble transferrin receptor and ferritin in iron deficiency and chronic disease," Scandinavian Journal of Clinical & Laboratory Investigation, vol. 65, pp. 571–576, 2005.

[25] K. Thorstensen and I. Romslo, "The transferrin receptor: its diagnostic value and its potential as therapeutic target," Scandinavian Journal of Clinical & Laboratory Investigation, Supplement, vol. 215, pp. 113–120, 1993.

[26] E. Chua, J. E. Clague, A. K. Sharma, M. A. Horan, and M. Lombard, "Serum transferrin receptor assay in iron deficiency anemia and anaemia of chronic disease in the elderly," QJM: An International Journal of Medicine, vol. 92, no. 10, pp. 587–594, 1999.

[27] Y. J. Shih, R. D. Baynes, B. G. Hudson, C. H. Flowers, B. S. Skikne, and J. D. Cook, "Serum transferrin receptor is a truncated form of tissue receptor," The Journal of Biological Chemistry, vol. 265, no. 31, pp. 19077–19081, 1990.

[28] R. D. Baynes, G. K. Reddy, Y. J. Shih, B. S. Skikne, and J. D. Cook, "Serum form of the erythropoietin receptor identified by a sequence- specific peptide antibody," Blood, vol. 82, no. 7, pp. 2088–2095, 1993.

[29] T. R. Daniels, T. Delgado, J. A. Rodriguez, G. Helguera, and M. L. Penichet, "The transferrin receptor part I: biology and targeting with cytotoxic antibodies for the treatment of cancer," Clinical Immunology, vol. 121, no. 2, pp. 144–158, 2006.

[30] B. Manger, A. Weiss, K. J. Hardy, and J. D. Stobo, "A transferrin receptor antibody represents one signal for the induction of IL-2 production by a human T cell line," Journal of Immunology, vol. 136, no. 2, pp. 532–538, 1986.

[31] P. Lira, A. Foradori, G. Grebe, and P. Vela, "Iron and folate deficiency in pregnant women at term," Revista Medica de Chile, vol. 112, no. 2, pp. 127–131, 1984.

[32] F. Mardones, A. Rioseco, M. Ocqueteau et al., "Anemia in pregnant women from the community of Puente Alto, Chile," Revista Medica de Chile, vol. 131, pp. 520–525, 2003.

[33] M. Munoz, J. A. Garcia-Erce, A. F. Remacha et al., "Deficiency of iron metabolism. Part II: iron deficiency and iron overload," American Journal of Clinical Pathology, vol. 121, no. 2, pp. 144–158, 2006.

[34] F. M. Torti and S. V. Torti, "Regulation of ferritin genes and protein," Blood, vol. 99, no. 10, pp. 3505–3516, 2002.

[35] M. T. Bertero and F. Caligaris-Cappio, "Anemia of chronic disorders in systemic autoimmune diseases," Haematologica, vol. 82, no. 3, pp. 375–381, 1997.
[36] A. N. Baer, E. N. Dessypris, and S. B. Krantz, “The pathogenesis of anemia in rheumatoid arthritis: a clinical and laboratory analysis,” *Seminars in Arthritis and Rheumatism*, vol. 19, no. 4, pp. 209–223, 1990.

[37] G. Vreugdenhil, C. A. Baltus, H. G. van Eijk, and A. J. G. Swaak, “Anaemia of chronic disease: diagnostic significance of erythrocyte and serological parameters in iron deficient rheumatoid arthritis patients,” *British Journal of Rheumatology*, vol. 29, no. 2, pp. 105–110, 1990.

[38] D. L. Witte, D. S. Angstadt, S. H. Davis, and R. D. Schrantz, “Predicting bone marrow iron stores in anemic patients in a community hospital using ferritin and erythrocyte sedimentation rate,” *American Journal of Clinical Pathology*, vol. 90, no. 1, pp. 85–87, 1988.

[39] T. Chijiwa, K. Nishiya, and K. Hashimoto, “Serum transferrin receptor levels in patients with rheumatoid arthritis are correlated with indicators for anaemia,” *Clinical Rheumatology*, vol. 20, no. 5, pp. 307–313, 2001.

[40] A. F. Remacha, M. P. Sarda, M. Parellada, J. Ubeda, and R. Manteiga, “The role of serum transferrin receptor in the diagnosis of iron deficiency,” *Haematologica*, vol. 83, no. 11, pp. 963–966, 1998.

[41] B. S. Skikne, C. H. Flowers, and J. D. Cook, “Serum transferrin receptor: a quantitative measure of tissue iron deficiency,” *Blood*, vol. 75, no. 9, pp. 1870–1876, 1990.

[42] M. M. Zorgati, R. Hafsia, A. Bahlous, S. Bahri, and S. Ben Ammar, “Soluble tranferrin receptor in the biological diagnosis of iron deficiency. Report of 24 cases,” *La Tunisie Médicale*, vol. 87, no. 12, pp. 818–823, 2009.

[43] S. J. Thorpe, A. Heath, G. Sharp, J. Cook, R. Ellis, and M. Worwood, “A WHO Reference Reagent for the Serum Transferrin Receptor (sTfR): international collaborative study to evaluate a recombinant soluble transferrin receptor preparation,” *Clinical Chemistry and Laboratory Medicine*, vol. 48, no. 6, pp. 815–820, 2010.