SERUM ERYTHROPOIETIN AND ERYTHROID ACTIVITY IN VITAMIN B$_{12}$ DEFICIENCY

ANGEL F. REMACHA, MAR BELLIDO, FATIMA GARCÍA-DIE, NURIA MARCO, JOSEP UBEDA, ENRIC GIMFERRER

Hematology Department, Hospital de la Santa Creu i Sant Pau, Barcelona, Spain

ABSTRACT

We studied erythropoiesis in 31 patients with vitamin B$_{12}$ deficiency by measuring serum erythropoietin (s-Epo), serum transferrin receptor (s-TfR, taken as an index of total erythroid activity), reticulocyte count, and the reticulocyte maturation index (RMI). s-Epo and s-TfR were measured with commercial immunoassays, whereas reticulocyte count and RMI were determined by flow cytometry. s-Epo (123±196 U/L) and s-TfR (4.1±2 mg/L) levels were increased in patients with vitamin B$_{12}$ deficiency. The absolute reticulocyte counts were decreased (29±18×10^9/L) with a relative increase in the most immature fractions (RMI: 29.6±18%). A significant negative relationship was found between s-Epo and Hb level (r = –0.65, p < 0.0001). On the average, however, s-Epo was inappropriately low for the degree of anemia, since the observed/predicted (O/P) s-Epo ratio was 0.80±0.28 in vitamin B$_{12}$ deficiency vs 1.00±0.16 in a group of patients with iron deficiency anemia. It is concluded that at least a portion of patients with vitamin B$_{12}$ deficiency have serum erythropoietin levels that are inappropriately low for the degree of anemia.

©1997, Ferrata Storti Foundation

Key words: vitamin B$_{12}$, reticulocyte, erythropoietin, transferrin receptor

I n recent years new tools have been developed for investigating erythropoiesis. These new parameters include reticulocyte count, serum erythropoietin (s-Epo) and transferrin receptor (s-TfR).[1,2] In addition, flow cytometry has simplified and improved the determination of the reticulocyte count.[3] In the present work we used these tools to assess the state of erythropoiesis in vitamin B$_{12}$ deficiency.

Patients and Methods

Thirty-one patients (15 women and 16 men, aged between 18 and 76 years) with vitamin B$_{12}$ deficiency were evaluated. The cause of B$_{12}$ deficiency was pernicious anemia in 24 cases, gastrectomy in 6 and ileal malabsorption in 1 case. The presence of concomitant iron deficiency and/or renal failure was excluded in all patients (all cases with B$_{12}$ deficiency had serum ferritin >30 μg/L and serum creatinine < 100 μmol/L).

At the time of diagnosis serum levels of Epo (s-Epo) and of TfR (s-TfR) were measured using commercial immunoassays (COAT-RIA, Bio-Mérieux, Lyon, France, and AMGEN, Thousand Oaks, CA, USA, respectively). Reticulocyte count and maturity fractions (HFR, MFR and LFR) were studied by flow cytometry (Sysmex R-2000, Toa Ltd, Kyoto, Japan). The reticulocyte maturation index (RMI) was calculated using the different reticulocyte maturity fractions [RMI= 100(HFR+MFR)/LFR]. Standard hematological cell counts were obtained using a cell counter (Technicon H-3, Bayer Diagnostics, Münich, Germany).

The values obtained for these parameters were compared with a reference population made up of 140 healthy persons in the case of reticulocytes, of 63 for s-TfR and of 76 for s-Epo. Furthermore, the levels of s-Epo in B12 deficiency were compared with those of a group of patients with iron deficiency anemia (n = 77).

The results were expressed as mean±standard deviation and maximum and minimal values. The Student´s t-test was employed to compare the values obtained with those of the reference group. Relationships between the parameters were investigated by simple and multiple regression analysis. The regression plots were compared by evaluating the coincidence of slopes and intercepts.[4,5] In the case of s-Epo, the observed/predicted (O/P) ratio of s-Epo was determined using the predicted values calculated by the regression analysis and the measured (observed) levels of s-Epo.[1] The O/P ratio in B$_{12}$ deficiency and iron deficiency anemia was compared by a Student´s t test.

Results and Comments

Results are summarized in Table 1. s-Epo (123±196 U/L) and s-TfR (4.1±2 mg/L) levels were increased in patients with vitamin B$_{12}$ deficiency. The absolute reticulocyte counts were decreased (29±18x10^9/L) with a relative increase in the most immature fractions (RMI: 29.6±18%). A significant negative relationship was found between s-Epo and Hb level (r = –0.65, p < 0.0001). However, 7 out of the 31 cases showed an O/P ratio for s-Epo that was lower than the expected lower normal value (0.7).

The s-Epo levels found in vitamin B$_{12}$ deficiency were compared with those of a group patients with iron deficiency anemia having similar degrees of anemia. As expected, in iron deficiency anemia

	Vitamin B12 deficiency ± 1 SD (range)	Reference subject	Comparison
Hb (g/dL)	8.4±3.4 (3.5-15.1)		
MCV (fl)	114±12 (95-139)		
B12 (pmol/L)	38±35 (0-129)	150-1150	
Red cell folate (nmol/L)	732±262 (290-1378)	560-2430	
s-Ftn (ng/L)	229±262 (30-1112)	M:32-350 F:13-150	
s-Epo (U/L)	123±196 (2-850)	7.7±4.4 (2-18)	t=3.29 p=0.003
s-TfR (mg/L)	4.1±2 (1.8-10)	2.6±0.5 (1.5-3.7)	t=3.94 p<0.0001
Reticulocyte count x10^9/L	29±18 (3-80)	44±19 (14-74)	t=-4.16 p<0.0001
RMI (%)*	29.6±18 (5.3-82.2)	9.3±4.6 (2.7-18.3)	t=6.11 p<0.0001

Table 1. Hematological data from vitamin B12 deficient patients.

*RMI, reticulocyte maturation index = (HFR+MFR) x 100/LFR.

there was a close inverse relationship between Hb and s-Epo (r= –0.94, p<0.0001); however, there was a significant difference between the regression curve of log s-Epo versus Hb in iron deficiency anemia and that in B12 deficiency (iron deficiency anemia: log s-Epo = 3.94-0.0236 Hb; vitamin B12 deficiency: log s-Epo = 3.23-0.02 Hb) (p < 0.00001). At any given Hb level, s-Epo values were lower in patients with vitamin B12 deficiency than in those with iron deficiency.

The above findings indicate that serum erythropoietin was inappropriately low for the degree of anemia in patients with vitamin B12 deficiency as compared with those with iron deficiency. About one fifth of the patients with a vitamin B12 deficiency showed values for O/P ratio typical of the condition defined as defective endogenous erythropoietin production.[5] This was previously observed by Carmel and MacPhee.[6] In their study, of 21 cobalamin-deficient patients with anemia, the 4 least anemic ones showed inappropriately low erythropoietin levels. In addition, although erythropoietin levels correlated with the severity of the anemia, wide individual variations were observed.

The reason(s) for the inappropriately low s-Epo levels in vitamin B12 deficiency are not clear. Low levels in patients with expanded erythropoiesis may reflect an increase in the rate of utilization by a proliferating pool of erythroid cells.[5] On the other hand, Lezon et al.[7] found that there is an inverse relationship between the rate of stimulated erythropoietin production and erythropoietic marrow activity. Inappropriately low s-Epo levels have also been observed in thalassemia intermedia.[8]

The shift in reticulocyte fractions was also interesting. Absolute counts showed no increase despite the rise in immature reticulocytes (high RMI). This pattern of reticulocyte count may reflect ineffective erythropoiesis.

References

1. 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.
2. Beguin Y. The soluble transferrin receptor: biological aspects and clinical usefulness as quantitative measure of erythropoiesis. Haematologica 1992; 77:1-10.
3. Spanish multicentric study group for hematopoietic recovery. Flow cytometric reticulocyte quantification in the evaluation of hematologic recovery. Eur J Haematol 1995; 53:293-7.
4. Barosi G, Cazzola M, De Vincentiis A, Grossi A, Tura S. Guidelines for the use of the recombinat human erythropoietin. Haematologica 1994; 79:526-33.
5. Cazzola M. Beguin Y. New tools for clinical evaluation of erythron function in man. Br J Haematol 1992; 80:278-84.
6. Carmel R, MacPhee RDJr. Erythropoietin levels in cobalamin deficiency: comparison of anemic and non-anemic, subtly deficient patients. Eur J Haematol 1992; 48:159-62.
7. Lezon C, Alippi RM, Barcelo AC, Martinez MP, Conti MI, Bozzini CE. Depression of stimulated erythropoietin production in mice with enhanced erythropoiesis. Haematologica 1995; 80:491-4.
8. Dore F, Bonfigli S, Gaviano E, Pardini S, Longinotti M. Serum transferrin receptor levels in patients with thalassemia intermedia during rHuEpo administration. Haematologica 1996; 81:37-9.