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SERUM ERYTHROPOIETIN IN THE DIAGNOSIS OF POLYCYTHEMIA VERA. A FOLLOW-UP STUDY

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ABSTRACT

Background and Objective. It has been suggested that the determination of serum erythropoietin (sEpo) may be useful in distinguishing between polycythemia vera (PV), relative polycythemia and secondary polycythemia (SP), but no conclusive evidence has yet been provided for this. In the present work, we evaluated the role of sEpo in the differential diagnosis of polycythemia vera and its usefulness in the follow-up of PV patients.

Methods. sEpo was assessed in 190 patients with polycythemia of different etiologies. A follow-up study was carried out in some of these patients (27 with secondary polycythemia and 17 with polycythemia vera).

Results. sEpo levels were higher in SP than in PV and relative polycythemia. There were, however, differences with regard to the various etiologies of SP. Polycythemia related to congenital heart disorders showed the highest levels of sEpo of the SP. When a study was conducted, sEpo alone as a diagnostic parameter displayed an efficiency of more than 90% and the most discriminating value was 5 U/L. Using lower levels (below 2 U/L) and higher levels (above 12 U/L), it was possible to distinguish between SP and PV, although an important overlap was detected between these limits (approximately 50% of cases). The follow-up study showed that in half the patients with SP the levels of sEpo were at times < 12 U/L and at other times greater than this value. At least three determinations were necessary to detect an elevated reading. In PV after venesection there was an increase in sEpo in some cases, although most of the time there was no change.

Interpretation and Conclusions. Using sEpo, it was possible to differentiate between PV and SP, despite an important overlap. A follow-up study demonstrated that the increase in sEpo was intermittent in SP and that in many of these cases more than one determination could be helpful.

Key words: erythropoietin, erythrocytosis, polycythemia vera, red cell

The *Polycythemia Vera Study Group* (PVSG) criteria[1,2] are classically used to diagnose the myeloproliferative disorder known as *polycythemia vera* (PV). These criteria are very specific (almost 100%), despite having a sensitivity of approximately 70%.[3,4] It is therefore not easy to ascertain the cause of polycythemia in 19 to 30% of cases (idiopathic polycythemia). For this reason, the usefulness of other parameters not included in the PVSG criteria has been evaluated.[5-10]

Serum erythropoietin (sEpo) levels are affected by both red cell mass and erythropoietic activity.[11-13] Thus sEpo could be potentially helpful in distinguishing between the different types of polycythemia.[14-18] A decreased sEpo value could differentiate between PV and secondary polycythemia (SP),[18] although many authors have detected an important overlap between sEpo levels using biological and immunological tests.[19] Nevertheless, the role of sEpo in helping to classify polycythemic patients should be assessed through a complete diagnostic analysis, including receiver operating characteristic (ROC) curves, logistic analysis and grouping secondary polycythemias on the basis of etiology. Moreover, there have been very few studies on the follow-up of sEpo and on its possible usefulness in the management of polycythemia.

In the present work, we evaluated the role of sEpo in the differential diagnosis of polycythemia vera and its utility in the follow-up of PV patients.

Materials and Methods

Population characteristics

Serum erythropoietin was studied in 190 patients with polycythemia (43 PV, age: 61±12, range 30-83 years, sex: 27 males and 16 females; 20 relative polycythemia, and 127 SP, age: 51±16, range 16-90 years, sex: 90 males, 37 females). The various etiologies of SP included congenital heart disease in 24 cases, postrenal transplantation in 17, renal cysts in 15, smoker's polycythemia in 34 and pulmonary disease in 37.

Hb was above 17.0 g/dL in women and 17.5 g/dL in men. All cases of PV fulfilled PVSG criteria.[1,2] Reduced plasma volume was demonstrated in relative polycythemias.[20]

A follow-up study was carried out in 27 patients with SP and

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in 17 with PV. Only patients for whom three or more sEpo determinations were performed were included in the follow-up study. Blood cell counts and the number of phlebotomies were also recorded. As regards SP, we were especially interested in the changes in sEpo. For PV we studied the influence of venesection on the sEpo levels. A change was considered to be significant when the difference between two determinations was ≥ 4 U/L.

Methods

Serum Epo levels were measured with a commercial immunoassay (Coat-Ria, Bio-Merieux, Lyon, France). Hematological counts were evaluated at diagnosis and during the follow-up (Technicon, Bayer, Munich, Germany).

Statistics

Data were expressed as mean, standard deviation and maximum and minimum values. Variance analysis and an *a priori* contrast were employed to compare the values obtained for the different types of polycythemia, with Hb being used as a covariate. Log Epo was used to make these comparisons since the homogeneity test for variance of sEpo in the different groups was significant.

A diagnostic study was carried out using the values of sEpo in SP and PV. The study included a logistic regression to classify subjects with different levels of sEpo (SPSS-Win 5.02, Chicago, IL, USA) and a receiver operating characteristic curve (ROC) (GraphROC for Windows, Turku, Finland).

Sensitivity or true positive (y-axis) versus 1-specificity or false positive (x-axis) was represented in the ROC curves. The diagnostic value (sensitivity, specificity) could be obtained at every point or in every concentration. The area below the curve was calculated and used to compare the value of sEpo as a diagnostic tool along with other tests.[21]

Logistic regression provided an equation from which a probability was obtained for every observed sEpo level. The patient could be regarded as having PV or SP on the basis of this probability. Logistic regression also provided the odds ratio for sEpo.

Results

Comparison between polycythemias

There were significant differences between the different groups of polycythemia (analysis of variance of log sEpo, F=116.13, p<0.00001); log sEpo was higher in SP than in PV or in relative polycythemia (RP) [differences: SP vs PV = 30.2, 95% confidence interval (CI): 19.5-46.8 U/L; SP vs RP = 2.5, 95% CI: 1.3-4.5 U/L; RP vs PV = 12.4; 95% CI: 6.2-24.5 U/L].

There were also differences between the various types of SP. sEpo levels were higher in all types of SP than in PV (Table 1). Polycythemia associated with congenital heart disease showed the highest sEpo levels (difference = 93, 95% CI = 46-140 U/L); moreover, sEpo levels in postrenal transplantation polycythemia exceeded those of all other types of SP except that associated with congenital heart disease (difference = 43, 95% CI = 25-62 U/L). There were no differences in polycythemia owing to renal cysts, smoking or pulmonary disorders.

Diagnostic study

Serum Epo alone displayed an efficiency of more than 90% in the differential diagnosis between SP and PV. Analysis of the ROC curve (plotting true positives vs false positives) showed that the most discriminating Epo value was 5 U/L. However, there was an important overlap between sEpo levels in SP and PV. When very low (2 U/L or less) or high sEpo levels (12 U/L) were involved it was often possible to distinguish between SP and PV; nevertheless, the respective percentages of PV cases having sEpo values ≥ 2 U/L and SP cases with sEpo levels exceeding 12 U/L were 60% (26 out of 43) and 57% (72 out of 127 cases) (Figure 1).

A logistic regression showed that the differential equation was y (PV:1 and SP:0) = $1/1+e^{-}(2.65-0.59$ sEpo) (chi square = 119.3; p<0.0001). A value of sEpo could be incorporated in this equation and its probability calculated. When this value exceeds 0.5 PV is more probable. For example, if the sEpo level in a patient is 3 U/L, then y (the probability of being PV) = 0.71 and it would be more accurate to classify him as PV. By contrast, if the level were 9 U/L, then y=0.07, probably SP. The odds ratio for PV was 0.5531 (this is the value by which the odds of the y-variable is multiplied when the sEpo level increases 1 U/L).

Both studies demonstrated that 5 U/L was the threshold value for differentiating between the two types of polycythemia.

Table 1. Serum erythropoietin levels in patients with polycythemia.

	n.	*Hb (g/dL)*	*s-Epo U/L*	*s-Epo >12 u/L N (%)*	*s-Epo < 2 u/L N (%)*
Secondary polycythemias	127	18.4±1.1 (17-21.5)	47±111 (2-1000)	72 (56)	
Congenital heart disease	24	18.2±1.5 (17.6-21.5)	122±232 (3-1000)	18 (75)	
Postrenal transplantation	17	18±0.82 (17-21)	65±61 (5-193)	13 (76)	
Renal cysts	15	18.8±1.46 (17.5-2.04)	19±12 (5-44)	8 (53)	
Pulmonary disorders	37	18.1±0.93 (17-21)	22±26 (2-111)	19 (51)	
Smoker' polycythemia	34	18.3±1.14 (17-20)	23±36 (3-160)	14 (41)	
Relative polycythemia	20	17.5±0.34 (17-18.6)	10±4 (3-20)		
Polycythemia vera	43	185±12.9 (17.6-21.5)	2.2±2.6 (0-12)		26 (60)
Reference values	79	14±1.1 (12.5-16)	9±4 (2-18)		

N: no. of cases; %: percentage of cases; s-Epo: serum erythropoietin. Results of s-Epo are expressed as mean±SD; maximum and minimum values are in parentheses.

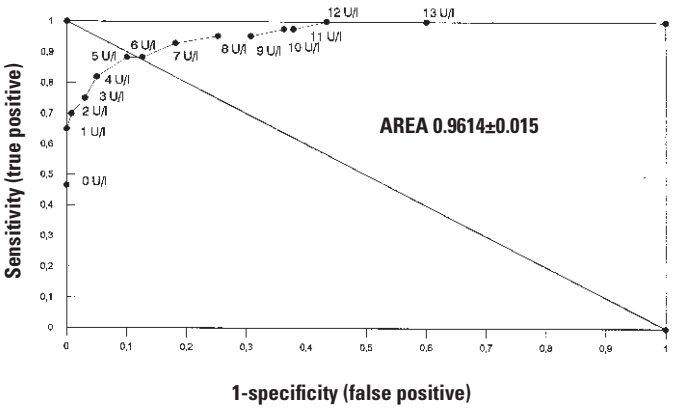

Figure 1. ROC plot of serum Epo in polycythemias. Specificity and sensitivity could be obtained for every concentration of s-Epo in the plot.

The follow-up study

For SP the follow-up study was carried out in 27 patients. In 4 of them (14.8%), the sEpo levels were below 12 U/L (limit of specificity), with no changes in the values being found at repeated sampling. In 12 cases (44.4%), the sEpo levels were always higher than 12 U/L. In 11 cases (40.7%), the sEpo levels were sometimes normal and on other occasions raised (Table 2). Al least three determinations were necessary to detect one with levels exceeding 12 U/L.

For PV 17 cases were evaluated as part of the follow-up study. In 4 of them the study was always performed during a polycythemic phase: sEpo was normal in 1 and always diminished (lower than 2 U/L) in the other 3 patients. In 13 cases sEpo levels were studied during polycythemia and after venesection: in 4 patients there was an increase in sEpo after venesection (the increase was ≥ 4 U/L) and in 9 cases there was no change after venesection (in 1 case sEpo was always higher than 2 U/L, and in 8 patients it was always diminished) (Table 3).

Discussion

Serum erythropoietin was found to be raised in SP and decreased in PV. The increase in sEpo was more marked in some types of SP than in others. In polycythemia associated with congenital heart disease and in that associated with postrenal transplantation, the values of sEpo exceeded those in the other types of SP. The role of sEpo in the diagnosis of polycythemia was assessed using a diagnostic study which included an ROC (receiver operator curve) plot and logistic regression analysis.

It was confirmed that the diagnostic efficiency of sEpo was high (> 90%) but its sensitivity and its specificity were only 100% when sEpo levels were extreme (lower than 2 U/L and higher than 12 U/L, respectively). Furthermore, there was a large overlap of values (40% of PV and 43% of SP fell within this range) and the percentage of patients with high sEpo levels varied according to the etiology of SP. The patients whose values were found in this range could have SP or PV.

In addition, attention should be drawn to a technical drawback of some importance. Low levels of sEpo put such a strain on the detection limit tech-

Table 2. Results of a follow-up study of serum erythropoietin levels in secondary polycythemia patients.

always normal EPO levels	*always high EPO levels*	*Variable s-Epo levels*
7/5/4 (20/19/19.5)	15/15/17 (17.4/18/17.6)	30/20/11 (18.7/18.6/18)
7/9/10 (17.7/17.5/18.2)	230/15/17/29 (19.8/20/20.6/19)	110/30/9/10 (18.9/19/18.8/19.2)
11/6/8 (18/18.2/18.9)	80/50/70 (19.7/21.1/19.8)	39/12/20 (18.5/19/18.8)
11/11/10 (17.9/19/18.5)	34/34/40 (17.6/18/18.6)	17/11/10 (20.5/20/19.8)
	32/19/22 (19.8/19/18.9)	17/8/15 (18/17.6/17.9)
	22/55/28 (20.7/19.9/21)	25/16/4 (19.1/18.8/17.5)
	80/50/53 (19.7/21.1/20.3)	24/12/9 (18/17.6/17.8)
	170/180/300/800 (17.9/18.8/17.9/19)	12/32/20 (18.3/17.5/17.9)
	420/600/750/900 (19.8/20/19/20.5)	8/11/26 (19.2/20/19.5)
	1000/1100/1200 (19/19.5/18.9)	21/45/8/9 (19.6/17.9/18.2/19.2)
	96/990/64 (17.3/18.5/17.4)	8/8/24 (18.5/18/18.5)
	70/250/120/220 (18.9/18.9/17.9/18.8)	

Results expressed as U/L. Each determination is separated by "/". Variable levels represent patients with at times high values (>12 U/L) and at other times normal values of serum erythropoietin (s-Epo). Hb levels in g/dL are in paretheses.

Table 3. Results of a follow-up study of serum erythropoietin levels in 17 PV patients.

No venesection s-EPO (U/L)	*Venesection no variation s-EPO (U/L)*	*Venesection with variation s-EPO (U/L)*
6/6.5/5 (20.3/20.3/19.8)	5/8* (18.7/15.1)	0/8* (19.7/15)
0/2/0 (18.9/19.5/19.2)	3/1* (19.9/15)	1/5* (18.8/13.9)
0/0/0 (19/19.5/19.2)	1/2.5* (18.7/13.5)	2/7* (18.7/14.5)
0/0/1 (19.8/18.9/19.6)	0/1* (18.9/15.5)	2/6* (18/14.2)
	2/0* (18.3/14.5)	
	0/0* (19.1/14.4)	
	0/0* (18.6/14)	
	0/0* (18.2/14.1)	
	0/0* (19.4/15.5)	

**Venesection controls. Each s-Epo determination is separated by "/". Every box represents a different case. Hb levels in g/dL are listed in parentheses.*

nique that the accuracy of the values becomes questionable.[22] In our experience the coefficient of variation (CV) is very high for low values of sEpo (in 20 samples with sEpo levels of 5 U/L or less, CV was 30±29, maximum-minimum values: 0-100%). By contrast, a high level (above 12 U/L) is not subject to this technical disadvantage and is, moreover, very specific.

Bearing the above in mind, studies were performed to determine whether sEpo levels varied during the follow-up period. It was seen that values were sometimes raised and at other times within the reference range in 44.5% of cases. In these patients follow-up is important since increased sEpo levels are only found in SP (in our experience after at least three determinations).

Some studies using accurate immunoassays have produced similar results concerning the role of sEpo in this disease (statistical differences between groups of polycythemias despite an important overlap),[16-19,23-25] but only a few of these focused on the follow-up period and on the evaluation of SP with reference to its etiology.[14,16] Our investigation supports the view that in approximately half the patients with polycythemia a follow-up study could increase the percentage of those with high levels of sEpo and thus help in their classification.

In order to explain the results obtained, it is necessary to bear in mind some aspects of Epo physiology.[11] The production of Epo is regulated by hypoxia, which is related to red cell mass. If hypoxia occurs, Epo is produced and there is a resulting increase in the number of red cells; this increase in red cells is usually able to correct the hypoxia, in which case Epo production ceases.[24] Although these aspects of Epo regulation are important for evaluating the behavior of the hormone in polycythemia, other mechanisms could also be involved.[12,26]

Two additional observations were provided by the follow-up study of sEpo in PV. First, sEpo levels did not vary during the polycythemic phase. In PV, the increased Hb level suppresses Epo production and keeps sEpo values low. However, studies in hypertransfused animals and in PV have demonstrated that it is impossible to totally suppress the endogenous production of Epo in most cases. This could explain why sEpo levels within the reference range were found in several PV patients.[27] Second, after venesection sEpo levels increased in one half of the patients, but did not reach levels higher than 12 U/L. Therefore in many cases the feedback control of Epo production is maintained and there is only a small increase in the hormone after venesection. Low sEpo levels (≤ 12 U/L) after venesection are very specific for PV.

In conclusion, sEpo determination is helpful in the differential diagnosis of polycythemias despite a large area of overlapping values. Only high levels of sEpo were capable of distinguishing satisfactorily between PV and SP (100% specificity), but only 50% of SP patients fulfilled this criterion. It is worth noting therefore that the increase in sEpo in SP is intermittent and that a follow-up study could be helpful in differentiating between SP and PV.

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