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# *Individual differences in the response to recombinant human erythropoietin therapy*

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## DIFERENCIAS INDIVIDUALES EN LA RESPUESTA A LA ERITROPOYETINA HUMANA RECOMBINANTE

### RESUMEN

*Se administró eritropoyetina por vía subcutánea a* **20** *enfermos en prediálisis y 21 en hemodiálisis. La anemia mejoró en todos los casos, si bien se observaron importantes diferencias individuales en cuanto al ritmo de ascenso de la hemoglobina. Del análisis de los factores potencialmente reponsables de las diferencias individuales no se encontraron diferencias entre ambos grupos de enfermos. Ni la edad, peso, ni los niveles séricos de creatinina, hierro, ferritina, transferrina o los índices de saturación de la transferrina tuvieron influencia significativa sobre el ritmo de incremento de la hemoglobina. Se encontró una correlación negativa estadísticamente significativa entre los niveles basales de hemoglobina y eritropoyetina y el ritmo de incremento de la hemoglobina. La respuesta al tratamiento con eritropoyetina dependió también de la nefropatía de base. Los enfermos con glomerulonefritis y nefropatía de los Balcanes respondieron mejor al tratamiento con eritropoyetina que aquellos con nefropatías tubulointersticiales distintas de la nefropatía de los Balcanes. Esta mejor respuesta al tratamiento con eritropoyetina en enfermos con determinados procesos está de acuerdo con la correlación negativa previamente establecida entre los niveles iniciales de hemoglobina y eritropoyetina y la respuesta al tratamiento.*

Palabras clave: ***Eritropoyetina humana recombinante. Enfermos en predialisis. Enfermos en hemodialialis. Respuesta terapéutica.***

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**SUMMARY**

*Recombinant human erythropoietin (rHuEpo) was administered subcutaneously to 20 predialysis and 2 1 hemodialysis (HD) patients. Anemia improved in all patients but individua/ differences in the rate of hemoglobin (Hb) increase were noted. The analysis of the factors possibly responsible for the individual differences in the response to rHuEpo therapy revealed the same factors correlating with Hb increase rate in both predialysis and HD patients. Patient age, body weight, serum creatinine, iron, ferritin, transferin levels and transferin saturation had no significant influente on the rate of Hb increase. Statistically significant negative correlation between initial Hb and erythropoietin levels (Epo) and Hb increase rate was found. The response to rHuEpo therapy also depended on the underlying kidney disease. Patients with glomerulonephritis (GN) and Balkan endemic nephropathy (BEN) responded better to rHuEpo therapy than those with tubulointerstitial nephropathy other than BEN. This better response to rHuEpo therapy* in *patients with GN and BEN is in accordance with already established negative correlation between the initial both Hb and Epo levels and response to rHuEpo therapy.*

Key words: ***Recombinanf human eryfhropoiefín. Predialysis patienfs. Hemodialysis pafienfs. Therapy response.***

## Introduction

Human recombinant erythropoietin (rHuEpo) is widely used for correcting anemia of chronic renal failure (CRF) patients. Initial studies have been carried out in patients on regular hemodialysis [1-3], but successful results in predialysis CRF patients [4-7], CAPD patients [8] as well as in patients with kidney graft failure [9] were reported. Anemia improvement was reported in majority of patients treated with rHuEpo. Nevertheless, individual differences in the rate and degree of the improvement were noted but not entirely elucidated [3,6,7,10,11].

The principal objective of this study was to analyze the factors possibly responsible for the individual differences in the response to rHuEpo therapy.

## Patients and methods

### *Patients*

The study comprised 20 patients with CRF not yet requiring dialysis (predialysis patients) and 21 patients with end-stage renal disease on maintenance hemodialysis (HD patients). The predialysis patients group consisted of 13 women and 7 men with stable serum creatinine levels between 315 to 835 µmol/l. The diagnosis of renal disease was established by clinical and laboratory criteria and renal biopsy in patients with glomerulonephritis (GN). Primary renal disease was: mesangiocapillary CN in 2, focal segmental glomerulosclerosis (FSG) in 2, Balkan endemic nephropathy (BEN) in 5, reflux nephropathy in 2, pyelonephritis in 2, polycystyc kidney disease in 4 and hypertensive nephrosclerosis in 3. Twenty-one HD patients, 14 women and 7 men, on regular hemodialysis for 13 to 210 months (71 .6 ± 58.8) were dialyzed three times a week for 4 hours using cuprophan membrane dialyrers. Diagnosis of their renal disease included GN in 5 (mesangiocapillary 1, FSG 3, membranous I), BEN in 4, reflux nephropathy in 3, pyelonephritis in 3, hypertensive nephrosclerosis in 2, nephrolithiasis in 2, while 2 patients were anephric.

All patients suffered anemia with hemoglobin (Hb) level less than 82 g/l at the outset of the rHuEpo therapy. The exclusion criteria for the study were: anemia due to causes other than CRF, uncontrolled hypertension, uncontrolled diabetes mellitus, abnormal liver function, pregnancy, therapy known to affect erythropoiesis, folate, $B_{12}$ or iron deficiency.

The study was approved by the Ethics Commitee of the University Clinical Center. Written informed consent was obtained from each patient.

### *Study Design*

The rHuEpo used was kindly given by Cilag AG International and it was subcutaneously administered. The starting dose of 50 U/kg body weight (bw) rHuEpo three times a week was maintained for four weeks. As the target Hb level of 100-120 g/l had not been achieved at the end of four-week period, the dose was increased by 25 U/kg bw. When the target Hb level was achieved the rHuEpo dose was regu-

larly adjusted to maintain a Hb level of about 100 g/l. The patients were followed up for 3 to 26 months.

The dietary protein intake was limited to 0,6 g/kg/day in predialysis patients, while normal protein intake was recommended to HD patients. Iron supplementation was given to patients with normal or lou serum iron level or ferritin levels 100 µg/l. Antihypertensive therapy was adjusted to keep blood pressure normal.

A complete blood count was done once a week during the first eight-weeks and once a fortnight later. Blood chemistry including urea, creatinine, electrolytes, liver function test, serum iron, transferin, transferin saturation, total iron binding capacity and serum ferritin was performed once a month.

Serum erythropoietin levels (Epo) were measured by radioimmunoassay at L.awrence Berkeley Laboratory( Berkeley, California in 15 patients at the outset of the study. The control value of serum Epo in healthy nonanemic volunteers was 17.8 ± (SD) 4.2 mU/ml [12].

Intact parathormon (PTH) plasma levels were determined by radio-immunodssay (Nichols Institute Diagnostics, normal range 10-53 pg/ml).

***Statistical analyses***

The rate of Hb increase during initial phase of rHuEpo therapy was calculated as follows:

Hb increase = Hbt – HbO/number of weeks until Hb, were Hbt is the first Hb value in the target Hb interval (100-120 g/l), HbO the Hb value at the outset of the study.

CRF progression prior to and during rHuEpo therapy was expressed by the slope of the regression line obtained by plotting the reciprocals of serum creatinine against time for these two periods [13]. The review periods were four to six months prior to therapy and three to 18 months under therapy. The number of observations per patients for each period ranged from 4 to 19.

The data were analyred using linear regression analysis, analysis of variance and Student t-test.

## Results

The results presented in table I show that HD patients had more severe anemia at outset of the study than predialysis patients. Treatment with rHuEpo improved anemia in all patients, but individual differences in the rate and the degree of Hb increase as well as in the rHuEpo doses necessary for reaching and maintaining the target Hb level were noted in both predialysis and HD patients. The target Hb levels reached in the fifth to twelfth week of therapy in all but two predialysis patients. In one patients iron deficiency due to patient irregular use of iron supplements was discovered. Another patient suffered pneumonia in eighth week of therapy wich caused anemia and renal function deterioration. Otherwise, the improvement of anemia due to rHuEpo therapy had no effect either on predialysis serum creatinine levels or on CRF progression expressed by the slope of regression line generated by plotting 1/serumcreatinine against time (table I).

**Table I.** Effects of rHuEpo therapy on anemia and serum creatinine levels

| | Patients | |
|---|---|---|
| | Predialysis | Hemodialysis |
| Number. | 20 | 21 |
| Age years. ..... | . 47.6 ± 13.3 | 43.6 ± 12.8 |
| rHuEpo dose U/kg/wk: | | |
| maximal ..... | 205.3 ± 49.0 | 196.1 ± 52.2 |
| – maintenance. ..... | . 70.6 ± 20.3 | 66.1 ± 32.8 |
| Hemoglobine g/l: | | |
| initial | 74.2 + 5.2 | 64.7± 9.2* |
| -at the end | 104.6+13.1 | **101.3+9.8** |
| No of weeks untill Hht | 7.1 ± 2.1 | 8.2 ± 2.5 |
| Hb increase, g/l/wk | 4.7 ± 1.9 | 4.6 ± 1.6 |
| s-creatinine umol/l: | | |
| initial | 508 ± 143 | 1013 ± 203 |
| – at the end | 690 ± 366 | 1069 ± 265 |
| slop 1/Cr VS time: | | |
| prior therapy | -0.111 ± 0.99 | |
| -during therapy | -0.103 ± 0,95 | |

rHuEp odos e maximal-dose necessary for reaching target Hb level (Hbt); Hb increase rate was calculate as dcscribe din Methods * p < 0.01 campared with predialysis patients.

The rate of Hb increase during the initial phase of rHuEpo therapy widely varied from 1.5 to 7.4 g/l/week. The coefficients of correlation between the rate of Hb increase and the factors wich might influence the response to rHuEpo therapy are presented in table II. The patient with iron deficiency and that

Table II. Coefficients of correlation between the rate of hemoglobin increase and the relevant variables

| variables | Predialysis patients | | Hemodialysis patients | |
|---|---|---|---|---|
| | r | P | r | P |
| | 0.088 | 0.364 | 0.098 | 0.672 |
| Body weight. ..... | 0.084 | 0.371 | 0.105 | 0.650 |
| Hb, initial ..... | -0.492 | 0.019 | -0.471 | 0.031 |
| Rt, initial ..... | 0.339 | 0.090 | 0.335 | 0.148 |
| Rt, maximal ..... | 0.496 | 0.021 | 0.507 | 0.019 |
| s-HuEpo dose * ..... | -0.499 | 0.018 | -0.423 | 0.063 |
| r-creatinine ..... | -0.249 | 0.160 | -0.354 | 0.118 |
| s-iron ..... | 0.195 | 0.219 | 0.335 | 0.148 |
| s-TIBC ..... | 0.142 | 0.303 | 0.196 | 0.42 1 |
| 5.transferin ..... | 0.058 | 0.409 | 0.102 | 0.661 |
| transferin saturatio n. | 0.255 | 0.323 | 0.212 | 0.355 |
| s-ferritin ..... | 0.286 | 0.125 | 0.335 | 0.148 |
| PT H..... | 0.332 | 0.090 | 0.350 | 0.119 |

Rt: retirulocyte.
* maxima, rHuEpo weekl ydos enecessar for rearhin target Hb level.

**Table III.** Mean (SD) serum iron, ferritin and transferin levels and intact PTH plasma levels in patients groups with different underlying kidney disease at the outset [1] and at the end [2] of the study

| Variables | GN | | BEN | | TIN | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 1 | 2 | 1 | 2 |
| s-iron, µmol/l | 16.3 (4.1) | 12.9 (1.8)* | 19.7 (8.0) | 16.1 (8.1) | 19.5 (9.1) | 15.3 (8.4) |
| s-ferritin µg/l | 184 (51) | 163 (20) | 196 (28) | 189 (21) | 216 (90) | 181 (67) |
| s-transferin, g/l | 2.1 (0.4) | 2.0 (0.3) | 1.9 (0.4) | 1.9 (0.3) | 2.0 (0.4) | 1.9 (0.4) |
| PTH, pg/ml | 92 (361 | | 89 (33; | – | 101 (39) | – |

GN: glomerulonephritis; BEN: Balkan endemic nephropathy; TIN: tubulointerstitial nephropathy other than BEN.
* $p < 0.05$ compared with initial value.

with pneumonia were excluded from this analysis. The results presented showed statistically significant negative correlation between initial Hb level and Hb increase rate in both patient groups examined. No significant correlation between age, body weight or serurn creatinine, PTH, iron, total iron binding capacity, ferritin, transferin level, transferin saturation and the rate of Hb increase was noted.

Searching futher for factors influencing different response to rHuEpo therapy we compared the Hb increase rate for paticnt groups formed according to underlying kidney disease (fig. 1). Only the groups consisting of more than five patients were compared. Hb increase rate was significantly higher for the patients with GN and BEN than for patients with tubulointerstitial nephropathy other than BEN (TIN) (reflux nephropathy and pyelonephritis). At the same time patients with GN and BEN had significantly lower initial Hb levels than patients with TIN. No significant difference in serum iron, ferritin, transferin levels as well as serum PTH levels was found between the three etiologically different groups (table III).

In fig. 1 the serum Epo levels for patients with different kidney disease are also presented. The results indicated that patients with BEN and GN, who resposded faster to rHuEpo therapy, had lower initial serum Epo levels than patients with TIN. That directed us to examine the relationship hetween initial serum Epo level and the rate of Hb *increase* during rHuEpo therapy At the outset of the study serum Epo level was measured in 15 patients including 12 presented in fig 1 . The regression analysis, which compared all these Epa levels to Hb increase rate during rHuEpo therapy, revealed significant negative linear correlation between these two variabies (fig. 2).

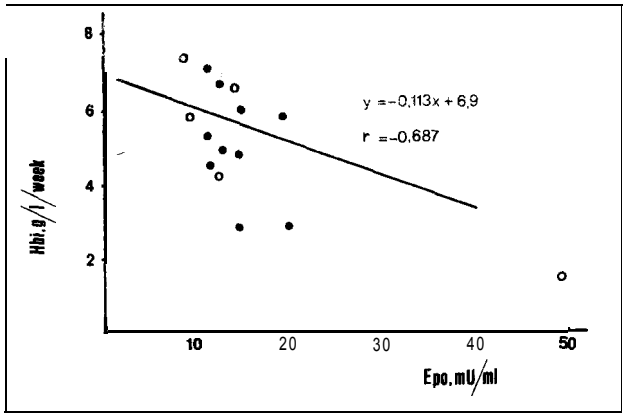


*Fig 2.-Relationship between the rate of hemoglobin increase during rHuEpo therapy (Hbi ) and serum erythropoietin levels (Epo) at the outset of the study.*
*o: predialysis patienh; • : hemodialysis patients*

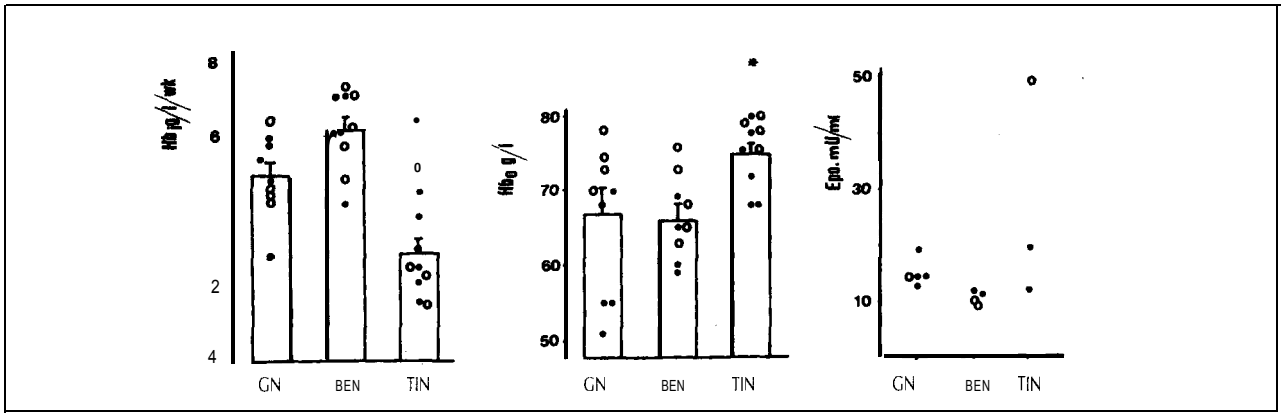


*Fig 1 .-The rate of hemoglobin increase during rHuEpo therapy (Hbi), initial hemoglobin values (Hbo) and initial serum immunoreactive Epo levels in predialysis (0) and hemodialysis (0) patients with different kidney disease.*

## Discusión

The correlation of anemia in predialysis and HD patients with rHuEpo therapy found here was expected as confirmed in earlier clinical studies [1-7]. The increase Hb level in the response to rHuEpo therapy has been shown to be dose dependent [4,5,14]. However, individual differences in the response to rHuEpo therapy was indicated in predialysis patients [6,7,11] as had previously been in HD patients [3,10,15].

This study has focused individual variations in the response to rHuEpo therapy. So far, several studies have reported the causes of resistance to rHuEpo therapy but only a few have discussed the factors which might influente rHuEpo therapy response. As the cause of resistance to rHuEpo therapy iron and vitamine deficiency [16,17], aluminium intoxication [18], hyperparathyroidism, acute or chronic inflammation [7] were reported. In two of our patients a poor response to rHuEDo therapy could be explained by iron deficiency or acute infection. In the remaining patients neither one of the causes of resistance to rHuEpo therapy was known. Niether one of predialysis patients was treated with aluminium hydroxid. Aluminium intoxication was not excluded in our HD patients but the dose of aluminium hydroxid in our patients was never higher than 1.6 gr/day. Therefore, aluminium intoxication most probably was not of influente on rHuEpo therapy response. Nevertheless, the significant individual differences in the Hb increase rate were noted. The statistical analysis revealed that the same factors correlated with the Hb increase rate during rHuEpo therapy in both predialysis and HD patients. Individual variations were not age, body weight or renal failure degree dependent. Absence of correlation between serum iron, ferritin, transferin level and transferin saturation at the outset of the study and the rate of Hb increase under rHuEpo therapy revealed that individual variations were not the results of different iron status in our patients.

In contrast to the other authors who found no correlation between underlying kidney disease and the response to rHuEpo therapy [6,10], our patients with GN and BEN responded better to rHuEpo therapy than those with TIN other than BEN. The difference in rHuEpo therapy response in patients with different underlying kidney disease was not caused by different iron status or degree of secondary hyperparathyroidism. In our previous studies patients with GN and BEN with advanced renal failure and those on maintenance HD had lower Hb and serum Epo levels than patients with pyelonephritis [20]. In the present study patients with GN and BEN had significantly lower initial Hb level than patients with TIN other than BEN. The better response to rHuEpo therapy in patients with GN and BEN was in accordance with the finding of significant negative correlation between initial Hb level and the response to rHuEpo therapy found here for the whole group examined. This correlation between initial Hb levels and rHuEpo therapy response contrasts with the results reported previously in hemodialysis [10] and predialysis [11] patients. Only Kleinman et al [6] reported the most rapid increase in hematocrit in two patients with the most severe anemia.

The higher rate of Hb increase in patients with lower initial serum Epo levels same as lower Hb values seems to be important but can not be explained by the results presented here. It could be suggested though that is due to the difference in sensitivity to Epo of erythroid precursors in the bone marrow of these patients.

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