Correlation Between Recombinant Human Erythropoietin Dose and Inflammatory Status in Dialysed Patients

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Once recombinant human erythropoietin (r-HuEPO) was introduced in daily practice, huge steps were made in combating the adverse effects induced by anemia in chronic kidney disease population. Still, r-HuEPO resistance and the doses ensuring the maximum therapeutic benefit remain matters of debate. The aim of our study was to assess the correlation between the presence and the degree of inflammation and the r-HuEPO requirements in chronic dialysis patients. We conducted a 2 years prospective study on 146 patients undergoing chronic dialysis treated with r-HuEPO. Based on their average CRP (C-reactive protein) levels, obtained from repeated samplings at 3 months interval, 3 groups were formed; we noted in each group the average values of r-HuEPO prescribed to achieve the optimum hemoglobin levels according to the dialysis best practice guidelines and all the adverse effects of the therapy. A direct correlation was observed between CRP levels and r-HuEPO requirements in the first 2 groups of patients (CRP under 6 mg/L and CRP values 6-20 mg/L), with significant increase in r-HuEPO doses between groups (p < 0.001); the third group, CRP values over 20 mg/dL, showed a minor, insignificant increase in average r-HuEPO doses compared to mild inflammation group (p = 0.199) and more adverse effects of the therapy (p < 0.05). Inflammation is an important determinant of anemia in chronic dialysis patients and can induce an increase in the doses of r-HuEPO. However, prescribing excessive r-HuEPO doses is not the answer in severe inflammatory status, due to lack of response and possible adverse effects.

Keywords: erythropoietin dose, inflammation, chronic dialysis

There is commonly known that anemia is one of the most important complications of chronic kidney disease (CKD) patients; its development represents mainly a consequence of renal impairment to synthesize erythropoietin (EPO) (fig. 1), and its severity is usually correlated with the degree of kidney dysfunction [1-10]. A significant decreased hemoglobin (Hb) levels in CKD patients represents an independent risk factor of cardiovascular events and progression to end-stage renal disease (ESRD) [2,5,11-19].

Since 1989, once the use of EPO-based erythropoiesis-stimulating agent (ESA) – recombinant human EPO (r-HuEPO) – for the treatment of anemia in CKD population became available, an important step forward was achieved by a better therapy control of anemia and the decrease of blood transfusions requirement and related complications (e.g.: transfusion-related lung injury, acute and chronic hemolytic adverse reactions, high risk of infectious diseases) [2,20,21]. R-HuEPO is a 59 kDa glycoprotein composed of a 484 amino acids chain, three tetra-antennary N-linked glycans (at Asn24,38,83) and one O-linked glycan at Ser 126 [21-23].

The improvement of the overall outcome through the correction of anemia by decreasing cardiovascular events rate and CKD progression is a general accepted notion in ESRD management; the optimal targeted levels of Hb are still, a matter of debate, since there are many studies that emphasize the risks of a complete anemia correction in dialysis patients [24]. Important trials concluded that both too low and too high (even high normal ranges) Hb levels are harmful, being associated with elevated risk of cardiovascular events through different functional maladaptation mechanisms: severe anemia induces hypoxia followed by systemic vasodilatation, increased cardiac output and finally left ventricular hypertrophy; augmented Hb concentration (biochemical translated as increased viscosity) is correlated with hypertension,

Fig. 1. Different causes of anemia in CKD patients

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endothelial impairment and consequently, vascular thrombosis [2,25]. Furthermore, high r-HuEPO doses are believed to have “toxic” pleiotropic effects, including the risk of developing retinopathy and neurotoxicity [26]. These modifications overlap with the chronic inflammation terrain well known to characterize maintenance dialysis patients, due to the up-rise of pro-inflammatory factors, and expressed by high levels of C-reactive protein (CRP), also associated with the presence of malnutrition expressed by hypoalbuminemia [27-31].

Considering all the above theories, there is a question that remains, yet, unanswered: is there a benefit in rising the r-HuEPO doses to correct the anemia in patients with high levels of inflammation, or, by doing this, we are only risking to add more pleiotropic adverse effects of high r-HuEPO doses without any benefit influence on hemocrit levels?

This study was aimed to assess the correlation between the presence/degree of inflammation in chronic dialysis patients and the EPO requirements and its effects on hemoglobin levels.

**Experimental part**

**Methods**

A 2 years prospective study was conducted on patients undergoing chronic dialysis (minimum dialysis vintage 6 months) in the Center of Dialysis of our hospital. We enrolled patients receiving r-HuEPO (epoetinum beta) for at least 6 months (inclusion criteria). The exclusion criteria were: known hematologic diseases, gastrointestinal bleedings, liver cirrhosis, active malignancies. All the patients considered for the study underwent laboratory investigations and imaging laboratory tests to certify complete diagnosis of anemia and allow evidence of other possible causes of anemia at the beginning of the research. The study group included 146 patients (17 on peritoneal dialysis and 129 on maintenance hemodialysis).

The chemical name of the r-HuEPO used is 1-165-Erythropoietin (human clone t HEPOFL 13 protein moiety), glycoform beta and its formula is: C809-H1301-N229-Erythropoietin (human clone rHEPOFL 13 protein moiety).

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Therefore, SC r-HuEPO administration was performed in doses are necessary using SC method [22,34,35].

Because of the slower absorption and elimination of r-HuEPO after SC (subcutaneous) versus IV (intravenous) administration (in which a rapid rate of elimination at a 50 IU/kg EPO dose and a peak plasma concentration of almost 1000 IU/L are noticed), a 30% decreased r-HuEPO doses are necessary using SC method [22,34,35]. Therefore, SC r-HuEPO administration was performed in all the patients included in our research.

Biochemical parameters were determined by routine laboratory techniques using an automated analyzer. Serum CRP and albumin levels were assessed every 3 months; for CRP levels, hospital’s laboratory has an upper normal limit of 6 mg/L. We divided the study cohort in 3 groups, considering the mean value of CRP obtained from all 6 determinations during the study period:

- **group 1** - CRP equal or lower than 6 mg/L;
- **group 2** - CRP values between 6-20 mg/L;
- **group 3** - CRP values above 20 mg/L.

We studied the distribution of average r-HuEPO requirements within these groups of patients; malnutrition was assessed based on serum albumin levels in each group (fig. 2). Furthermore, all the known adverse effects of r-HuEPO were specifically sought and noted (accelerated hypertension, thrombosis, flu-like symptoms).

**Statistical analysis**

For the assessed results, in the three groups of patients, correlation between the CRP levels and r-HuEPO doses were carried out. Skewness, Kurtosis and Shapiro-Wilk tests were performed to evaluate the distribution of data; additionally, one way ANOVA was performed. Statistical analysis was done using Excel and IBM SPSS Statistics v. 20.0. We also correlated average r-HuEPO doses in each group (1, 2, and 3) with the average albumin level in each of them.

**Results and discussions**

**Group 1** – 106 patients, CRP < 6 mg/L – consisted in 74 patients who were treated with an average dose of 2000 IU/week Epoetinum Beta (most of them on peritoneal dialysis or hemodialysis patients with polycystic kidney disease or chronic pyelonephritis), 30 patients with an average dose of 4000 IU/week and 2 patients with 5000 IU/week (patients with higher doses requirements due to chronic bleeding, other than gastrointestinal – urinary or prolonged arteriovenous fistula bleedings after dialysis punctures). The average group r-HuEPO dose was of 2622.64 IU (SD = 960.68 IU).

**Group 2** included 21 patients with acute periods of reversible inflammation that determined increased CRP mean values above normal (CRP 6-20 mg/L). The minimum average dose of r-HuEPO was 4000 IU/week, in patients with short term acute inflammation; we also recorded

![Fig. 2. The study design diagram](http://www.revistadechimie.ro)
The average group r-HuEPO dose was of 4428.57 IU (SD = 676.12 IU).

Group 3 consisted in 19 patients with significant chronic inflammation (CRP > 20 mg/L), presenting a minimum EPO dose of 4000 IU/week; we also observed a case requiring doses around 7000 IU/week. The average group EPO dose was of 4947.36 IU (SD = 235.37 IU).

35 patients associated different grades of malnutrition (23.28%). There were 16 cases of mild malnutrition, 15 of moderate malnutrition and 4 patients with severe malnutrition. Analysis of data revealed a relationship between the increase of r-HuEPO necessary and the degree of malnutrition, but the number of those with moderate and severe malnutrition was too small to make a comparison with statistical significance.

Regarding the Group 3 (patients with high CRP values), it revealed the lowest average serum albumin levels, significant reduced in comparison to Group 1 (p < 0.001) and Group 2 (p < 0.001). Albumin average values in Group 1 and 2 showed no differences.

A correlation has been made between the level of inflammation and the r-HuEPO doses in studied groups (fig. 3).

![Fig. 3. Necessary average r-HuEPO doses – correlated with the degree of inflammation](http://www.revistadechimie.ro REV .CHIM.(Bucharest)

A higher increase in r-HuEPO doses was attempted in 6 patients in group 3, with no response in hemoglobin levels and with noted adverse effects: 4 cases of accelerated hypertension requiring drug supplementation and 2 cases of vascular access thrombosis. No adverse effects known to be attributable to r-HuEPO were noted in Group 1 and only 2 patients with accelerated hypertension after rising the r-HuEPO doses were observed in Group 2.

In this study we aimed to demonstrate the existence of a correlation between erythropoietin requirements, the presence of chronic inflammation in patients on dialysis and the existence of a directly proportional relationship between the two.

Although there is clear evidence that anemia correction improves the quality of life and decreases the risk of mortality, some aspects should be considered when ESA are used in order to elevate Hb levels. In normal conditions, EPO is permanently synthesized (not stored) at a minimal degree of inflammation and the r-HuEPO doses were observed in Group 2.

The vast majority of dialysis patients show a chronic inflammatory status, possibly induced by an exacerbation of inflammatory mediators' synthesis due to macrophages and neutrophils activation [50-53]. Some studies have demonstrated that several biomarkers of inflammation like C-reactive protein (CRP) are closely associated with the presence of hypo-albuminemia as a marker of malnutrition [50,54]. This was the case also in our study: group 3 (severe inflammation) showed a significant decrease in average albumin values compared to group 2 (p = 0.199). There was a significant difference between the percentage of adverse effects noted in group 3 (31.5%, all remitted after dose decreased), compared to group 1 (0%), and group 2 (9.5%).

According to the statistical data, between the study groups 1 and 2 the average r-HuEPO doses showed a significant increase (p < 0.001); the calculated average r-HuEPO dose for group 3 was not different from group 2 (p = 0.199). There was a significant difference between the percentage of adverse effects noted in group 3 (31.5%, all remitted after dose decreased), compared to group 1 (0%), and group 2 (9.5%).
3 showed an insignificant increase, limited by the registered adverse effects and the lack of therapeutic effect.

Conclusions
In our study, each of the groups with higher CRP value, in ascending order, showed the elevation of the average EPO dose used during the 2 years period. It was noted that in the severe inflammation group we could not establish a direct relationship between the erythropoietin dose and the increase of inflammation. When CRP value is above 20, the erythropoietin requirements remained the same, with increasing doses it affects leading only to adverse effects of erythropoietin therapy.

Chronic inflammation is an important condition in maintenance dialysis patients. It affects the EPO necessary dose used during the 2 years period. It was noted that inflammation increases when CRP value is above 3. In the severe inflammation group we could not establish a relationship between the erythropoietin dose and the increase of inflammation. When CRP value is above 20, the erythropoietin requirements remained the same, with increasing doses it affects leading only to adverse effects of erythropoietin therapy.

References