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The Relation of Erythropoietin Towards Hemoglobin and Hematocrit in Varying Degrees of Renal Insufficiency

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ABSTRACT

Introduction: Hypoxia is a basic stimulant in production of erythropoietin (EPO). The primary function of erythrocytes is the transport of oxygen to tissues. Erythropoietin stimulates erythropoiesis which leads to increased production of erythrocytes- their total mass. This increases the capacity of the blood to carry oxygen, reduces the hypoxic stimulus and provides a negative feedback of stopping EPO production. The aim of this study was to establish a quantitative relationship between the concentration of erythropoietin, hemoglobin and hematocrit in different values of renal insufficiency. **Material and methods:** The survey was conducted on 562 subjects divided into two groups: with and without renal insufficiency. EPO, hemoglobin, hematocrit, serum creatinine and additional parameters iron, vitamin B12, and folic acid were determined by using immunochemical and spectrophotometric methods and glomerular filtration rate (GFR) was calculated as well. **Results:** EPO values (median) grow to the first degree of renal insufficiency, as compared to EPO values of healthy subjects, this increase is statistically significant, $p=0.002$. With further deterioration of renal function the values of EPO between all pathological groups are decreasing, and this decrease is statistically significant between first and second degree of renal insufficiency (RI) $p<0.001$. In the group of healthy subjects EPO is correlated $\rho = -0.532$, $p < 0.0005$ with hematocrit. The correlations are negative and strong and can be predicted by regression line ($EPO = 41.375 - Hct * .649$; $EPO = 61.41 - Hb * 0.355$). In the group of subjects with the first degree of renal insufficiency EPO is in correlation with hematocrit $\rho = -0.574$, $p < 0.0005$. It is also correlated with hemoglobin $\rho = -0.580$, $p < 0.0005$. The correlation is negative ($EPO = 42.168 - Hct * 0.678$). In the group of subjects with the third degree of renal insufficiency EPO is in correlation with hemoglobin $\rho = 0.257$, $p = 0.028$. The correlation is medium strong and positive. In the group of subjects with third and fourth degree of renal insufficiency EPO is not in correlation with hemoglobin and hematocrit $p > 0.05$. **Conclusion:** Renal dysfunction, depending on the level of RI effects differently on the biosynthesis of EPO in a diseased kidney, and consequently it also has a different effect on biosynthesis of HB in bone marrow and its content in the blood.

Key words: erythropoietin, hemoglobin, hematocrit, renal insufficiency and glomerular filtration rate

1. INTRODUCTION

Erythropoietin is a glycoprotein hormone composed of 165 amino acid residues with four complex carbohydrate chains attached with peptide in the four binding positions (MW 30.4 kDa), and it has the role of the primary regulator of erythropoiesis. EPO stimulates the proliferation and differentiation of erythroid precursor cells in the bone marrow and in this way it affects the production of red cells (1, 2).

Chronic renal insufficiency leads to hypo-regenerative anemia due to the lack of erythropoietin. The level of synthesis of EPO in the kidneys (or liver) is primarily governed by the needs of the given cells for oxygen. Anemia is one of the most common disorders in chronic renal insufficiency (3-7). Anemia

is diagnosed by measuring hemoglobin (Hb) levels (g / L) and hematocrit (Hct) (percentage of red blood cells in the blood) and by comparison with a given reference values. The most important reason of anemia is the inability of the kidneys to increase the synthesis of EPO in response to it (4, 8). For the normal maturation of erythrocytes, except erythropoietin, it is essential to have iron, folic acid and vitamin B12. Anemia is an independent risk factor for development of cardiovascular diseases in patients with chronic kidney disease. Large amount of studies that were conducted show that every tenth person in the world has a chronic kidney disease. Renal anemia is a consequence of chronic kidney disease and occurs at an early stage, it gets worse as the disease progresses (4, 5). With the fall

of the value of glomerular filtration rate the incidence of anemia is increased. In studies of patients with chronic kidney disease a direct correlation degree of anemia (concentration of Hb) and renal failure (6) has been demonstrated. The treatment for anemia with recombinant erythropoietin should be initiated on the basis of glomerular filtration rate and hemoglobin, but it is not enough clarified yet (7, 8). Apart from the mentioned facts, anemia in kidney patients is not recognized and treated at the right time; therefore this study has an aim to establish a quantitative relationship between the concentration of erythropoietin, hemoglobin and hematocrit in different values, glomerular filtration rate, and to determine the legality of these connections.

2. MATERIAL AND METHODS

The survey was conducted on 562 subjects divided into two groups with and without renal insufficiency. The subjects in the group with RI are divided into 4 stages according to GFR rate: first stage (60-89.99 ml / min / 1.73m²) (25%), second stage (30 to 59.99 ml / min / 1.73m²) 40%, third stage (15 to 29.99 ml / min / 1.73m²) and 20% of the fourth stage (\leq 14.99 ml / min / 1.73m²) 15% of the subjects. 365 (63%) subjects had renal insufficiency (RI) (male 174 (49%) and female 182 (51%)), while 206 (37%) were with healthy kidney function (male 104 (51%) and female 102 (49 %) (19)). The age structure of subjects with RI is on average 59 ± 16 years, whereas control group subjects are 49 ± 18 years. Additional parameters were set in order to exclude other types of anemia caused by deficiency of iron, vitamin B12 and folic acid. Humane samples were used for these tests: serum and whole blood. The values of erythropoietin, hemoglobin and hematocrit were determined. For the determination of Hb concentration the counter of blood elements was used (Cell Dyn 3700 system), which uses hemoglobin-hydroxylamine colorimetric method. Hematocrit was obtained by multiplying the volume of erythrocyte (MCV, mean corpuscular volume) with the number of red blood cells. Creatinine was determined by kinetic method based on Jaffe reaction on auto analyzer Dimension RxL Siemens. Erythropoietin is determined by enzymatic- chemiluminescent immunometric method on IMMULITE / IMMULITE 1000 (Siemens) (11). For each subject GF was calculated by using the calculator and applying the MDRD formula (Modification of Diet in Renal Disease). For the calculation we used the following variables: age, sex, and serum creatinine (12).

For statistical analysis of the data obtained we used software package SPSS for Windows (version 19.0, SPSS Inc., Chicago, Illinois, USA) and Microsoft Excel (version 11th of Microsoft Corporation, Redmond, WA, USA). For the connection and direction of the connection between the variables we used the correlation tests, depending on the type of variables (Spearman).

3. RESULTS

In the Table 1, values of EPO in subjects with first grade of renal insufficiency are different in male and female population and are statistically significant, $p = 0.002$.

EPO values were lower in men with average value of 12.25 mIU / mL, and a range (8.10 to 15.8 mIU / mL), than the value of EPO in women with average value of 16,6 mIU / mL range (13.4- 18.5 mIU / mL). Also, it was observed that with the weakening of kidney function the gender differences in EPO values is lost, $p > 0.05$. With the growth of the degree of renal insuf-

GF (ml/min/1,73m ²) Categories of RI	Gender	Number of subjects	Percentage (%)	p (c2)
$\geq 90,00$	Male	104	50.5	0.899
	Female	102	49.5	
	Total	206	100,0	
60,00–89,99	Male	43	48.9	0.831
	Female	45	51,1	
	Total	88	100.0	
30,00–59,99	Male	67	47.5	0.556
	Female	74	52,5	
	Total	141	100.0	
15,00–29,99	Male	34	46.6	0.558
	Female	39	53.4	
	Total	73	100,0	
$\leq 14,99$	Male	30	55.6	0.414
	Female	24	44.4	
	Total	54	100.0	

Table 1. Average values of EPO in all subjects, with and without renal insufficiency, in relation to gender.

Level of RI Glomerular filtration (ml/min/1.73m ²)		Spearman's rho	Hemato- crit (%)	Hemo- globin (g/L)
Healthy subjects (≥ 90.00)	EPO (mIU/mL)	Correlation Coefficient	-.532(**)	-.595(**)
		P	.0005	.0005
		N	206	206
1 st degree (60.00- 89.99)	EPO (mIU/mL)	Correlation Coefficient	-.574(**)	-.580(**)
		P	.0005	.0005
		N	88	88
2 nd degree (30.00- 59.99)	EPO (mIU/mL)	Correlation Coefficient	.083	.156
		P	.326	.065
		N	141	141
3 rd degree (15.00- 29.99)	EPO (mIU/mL)	Correlation Coefficient	.056	.257(*)
		P	.635	.028
		N	73	73
4 th degree (≤ 14.99)	EPO (mIU/mL)	Correlation Coefficient	.190	.208
		P	.169	.130
		N	54	54

Table 2. Correlations of EPO, hematocrit and hemoglobin in relation to the level of RI

ficiency EPO values (median) are decreasing, so the values of EPO from the second to fourth degree of RI are not statistically different between male and female subjects. Kruskal Wallis test showed that there is a statistical difference in the average values of EPO between certain categories of RI (in relation to the value GF), $p < 0.0005$. EPO values (medians) increase until the first degree of renal failure, as compared to EPO values of healthy subjects, this increase is statistically significant, $p = 0.002$.

In the group of healthy subjects (GF ≥ 90.0 ml / min / 1.73m²) EPO is correlated $\rho = -0.532$, $p < 0.0005$ with hematocrit. The correlation is strong and negative. With the fall of hematocrit values of EPO increase. In the same group, EPO is correlated with hemoglobin $\rho = -0.595$, $p < 0.0005$. The correlation is strong and negative. Value of EPO increases with the decrease of

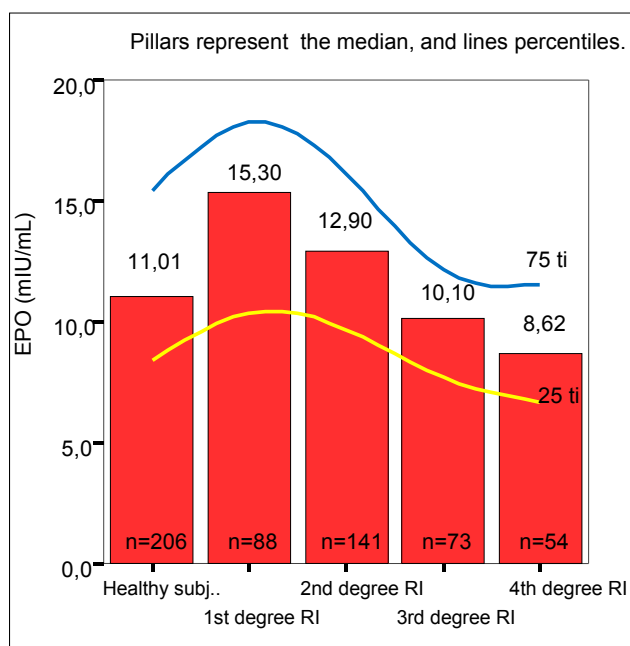


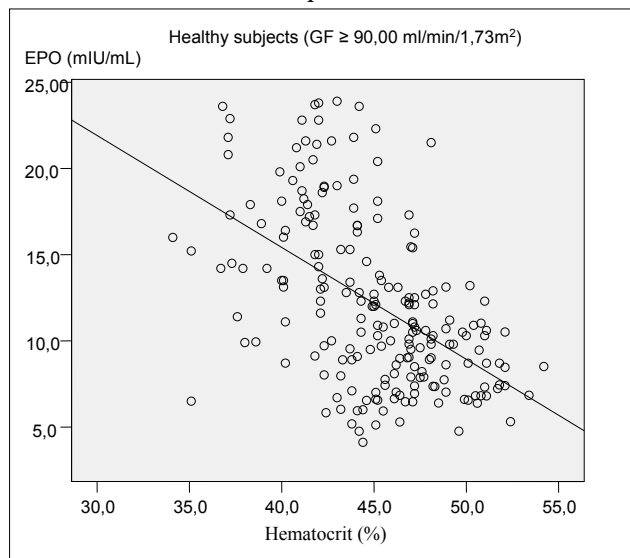
Figure 1. Distribution of individual erythropoietin with linear regression analysis (glomerular filtration ≥ 90 ml / min / 1.73m²) with hematocrit (healthy subjects)

hemoglobin. In the group of subjects with the first grade of RI (GF 60.00 to 89.99 ml / min / 1.73m²) EPO is correlated $\rho = -0.574$, $p < 0.0005$ with hematocrit. The correlation is strong and negative. The value of EPO increases with the decrease of hematocrit. In the same group, EPO is correlated with hemoglobin $\rho = -0.580$, $p < 0.0005$. The correlation is strong and negative. With the fall of hemoglobin the value of EPO increases.

In the group of subjects with third grade of RI (GF 15.00-29.99 ml / min / 1.73m²) EPO is correlated with hemoglobin $\rho = 0.257$, $p = 0.028$. The correlation is weak and positive. Hemoglobin decreases with the decrease of EPO and vice versa.

In the group of patients with second and fourth degree of renal insufficiency EPO is not correlated with hemoglobin and hematocrit, $p > 0.05$.

Figure 1 shows that in healthy subjects with normal renal function the values of EPO depend on hematocrit, $R^2 = 0.271$,



$p < 0.0005$. The dependence is linear, with the decrease of Pct the value of EPO increases, the dependence can be represented by linear regression equation: $EPO = 41,375 - Hct * .649$.

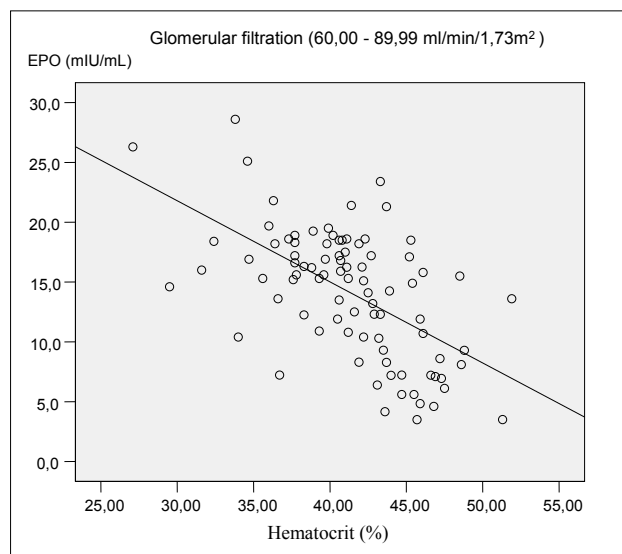


Figure 2. Distribution of individual erythropoietin levels and hematocrit with linear regression analysis with glomerular filtration (60.00-89.99 ml / min / 1.73m²)

Regression analysis of patients with renal insufficiency (60.00-89.99 ml / min / 1.73m²) in figure 2 showed a correlation between the levels of hematocrit and erythropoietin.

The dependence is linear and the value of EPO increases with the decrease in hematocrit values, this can be represented by linear regression equation: $EPO = 42,27 - Hct * 0.68$.

Regression analysis of healthy subjects in the figure 3 shows the correlation between the level of hemoglobin and EPO.

The dependence is linear, EPO value increases with the decrease in hemoglobin values, and this can be represented by linear regression equation: $EPO = 61.41 - Hb * .355$.

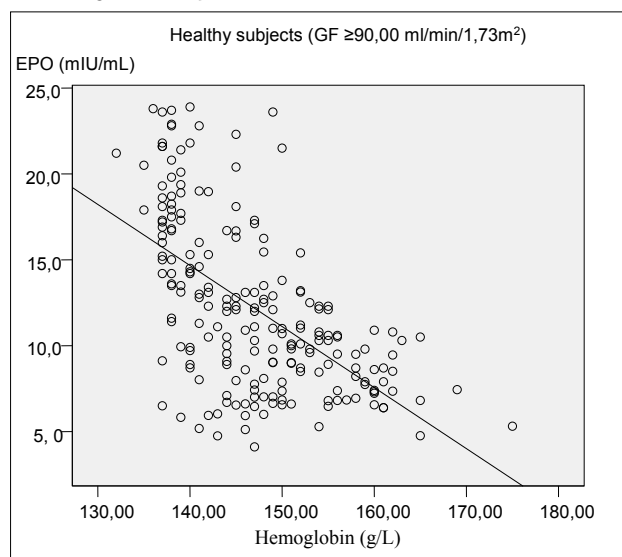


Figure 3. Distribution of individual erythropoietin and hemoglobin levels with linear regression analysis with glomerular filtration in healthy subjects (GF ≥ 90.00 ml / min / 1.73m²).

Regression analysis of patients with renal insufficiency (60.00-89.00 ml / min / 1.73m²) in figure 3, showed a correlation between the level of hemoglobin and erythropoietin.

The dependence is linear, EPO value increases with the de-

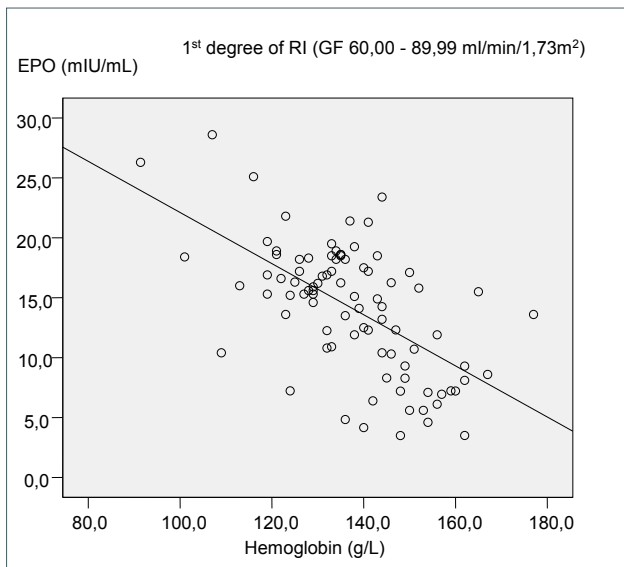


Figure 4. Distribution of individual erythropoietin and hemoglobin levels with linear regression analysis with glomerular filtration (60.00-89.00 mL / min / 1.73m²)

crease of hemoglobin values, and the dependence can be represented by linear regression equation: $EPO = 43,45 - Hb \cdot 0,21$.

Figure 5 shows that the value of EPO depends on the values of Hb in patients with the third degree of RI, $R^2 = 0,099$, $p = 0,007$.

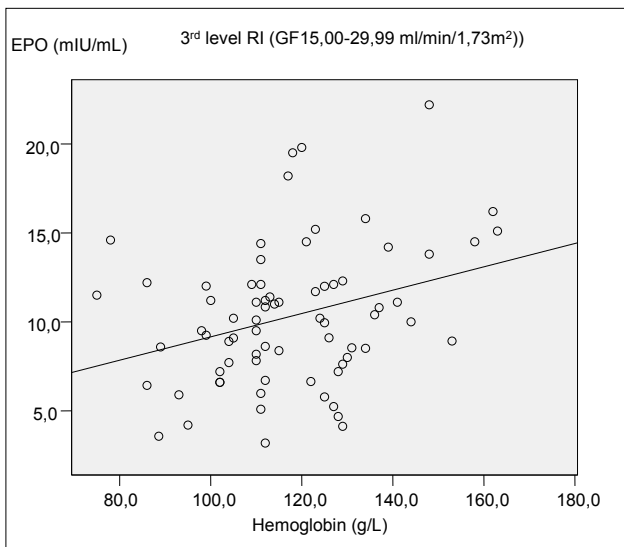


Figure 5. Distribution of individual erythropoietin and hemoglobin levels with linear regression analysis with glomerular filtration 15.00-29.99 ml / min / 1.73m²

The dependence is linear, EPO value decreases with the decrease of Hb values, and the dependence can be represented by linear regression equation: $EPO = 2,59 + 0,066 \cdot Hb$.

4. DISCUSSION

The importance as well as the frequency and severity of kidney disease obliges professional national organizations to issue recommendations for monitoring high-risk groups in order to discover the renal impairment as soon as possible and to plan a strategy for treating and monitoring of this disease, as well as educating the population and medical workers. It is very important to monitor the status and function of kidneys with certain diagnostic tests because adequate and timely treatment

can significantly slow the progression of renal failure and substantially preserve the function of kidneys (8, 10). Hyporegenerative anemia is a common manifestation of chronic renal failure and it is considered to be partly responsible for the symptoms of chronic fatigue and poor general health condition associated with chronic renal insufficiency. Timely detection of anemia in kidney patients is very important, and the importance of treating anemia is underlined as important fact in many recent studies, and it would reduce deaths (6, 9). In healthy people relationship between the mass of red blood cells and hemoglobin saturation with oxygen is linear. In chronically anemic people there is an inverse linear relationship between serum EPO and hemoglobin (13, 14). In healthy people, exposure to high altitude and consequent hypoxemia stimulates production of EPO (14, 15). Also, they speculate that this adjustment in the volume of red blood cells is similar to adjustments of inhabitants of high altitude to hypoxia (16). However, most authors report that the increase in the mass of red blood cells is inappropriate with regard to the degree of hypoxemia (17, 18).

Total amount of 562 subjects were included in this study, and they were divided into two groups, with (356) and without (206) renal insufficiency.

The level of erythropoietin is difficult to interpret in the context of renal failure. Three studies examined the levels of erythropoietin in relation to hemoglobin in patients with varying degrees of renal insufficiency. Radtke and colleagues measured EPO levels in 135 patients with renal failure (creatinine clearance between 2 and 90 mL / min) and found significantly increased average levels of EPO in all groups compared to the average level of EPO for 59 reference subjects ($CL_{cr} > 90$ mL / min). In patients with a creatinine clearance of <40 mL / min, EPO levels were not adequate to provide the degree of anemia. They found that levels of EPO are decreasing with increased deterioration of excretory renal function. However, the correlation between EPO and hemoglobin among various groups has not been investigated in this study (20). Korte and his associates have researched small groups of subjects (8 with moderate renal insufficiency and 9 reference subjects) and they found a significantly altered regulation (reduced secretion) of EPO in patients with moderate renal insufficiency (21). Fehr and colleagues measured EPO with 395 subjects and sought correlations between groups with different renal impairment. For subjects with creatinine clearance of <40 mL / min there was no evidence of correlation between the levels of EPO and hemoglobin compared to the reference group, and there was a significant inverse correlation between the levels of EPO and Hb in patients with a creatinine clearance above 40 mL / min $[EPO] (U / L) = 2,5 \times (140 [Hb] (g / L))$ or $[EPO] (U / L) = -2,5 \times [Hb] (g / L)$; below this clearance level, no significant correlation of EPO and hemoglobin was found (6). According to these inquiries we got the following results.

The highest EPO values in comparison to healthy subjects are in the first degree of RI, this difference is statistically significant $p=0,002$. With further increase of renal insufficiency, value of EPO decreases, between all categories of renal insufficiency, and the decline is statistically significant between second and third degree of renal insufficiency, $p < 0,0005$. The correlation searched for was between EPO, hematocrit and hemoglobin depending on the level of RI. The correlation between EPO and hematocrit was found in healthy subjects, EPO is correlated

$\rho = -0.532$, $p < 0.0005$ with hematocrit. The correlation is strong and negative. The value of EPO increases with the decrease of hematocrit. In referent group, EPO is in correlation with hemoglobin. The correlation is strong and negative. The value of EPO increases with the decrease of hemoglobin. With regression analysis of healthy subjects, correlations are presented with linear regression equation: $EPO = 61.41 - Hb \cdot .355$; $EPO = 41.37 - Hct \cdot 0.65$. The research on healthy subjects did not show a statistically significant effect of glomerular filtration rate on EPO value, $p > 0.05$. In the group of patients with the first grade of renal insufficiency EPO is correlated $\rho = -0.574$, $p < 0.0005$ with hematocrit. The correlation is strong and negative. The value of EPO increases with the decrease of hematocrit. In the same group, EPO is correlated with hemoglobin $\rho = -0.580$, $p < 0.0005$. The correlation is strong and negative. The value of EPO increases with the decrease of hemoglobin and this can be represented by linear regression equation: $EPO = 43.45 - Hb \cdot .213$. EPO is not correlated with hemoglobin and hematocrit ($p > 0.05$) in patients with second grade of RI. In patients with the third grade of renal insufficiency EPO is correlated with hemoglobin $\rho = 0.257$, $p = 0.028$. The correlation is weak and positive. Regression analysis showed a linear dependence in the third degree of RI. Value of EPO decreases with the decrease of hemoglobin and this dependence can be represented by linear regression equation: $EPO = 2.59 + Hb \cdot .066$. In the fourth degree of renal insufficiency EPO is not correlated with GF, hemoglobin or hematocrit, $p > .05$. Our results indicate that the reduced levels of erythropoietin synthesis are observed below GF (30ml / min / 1.73m²). Overall, the study showed that the evaluation of the regulation of erythropoietin occurs differently for different level of RI.

5. CONCLUSIONS

Renal dysfunction, depending on the level of RI responds differently to the biosynthesis of EPO in a diseased kidney, and consequently, it differently affects the biosynthesis of hemoglobin in the bone marrow and its contents in whole blood.

CONFLICT OF INTEREST: NONE DECLARED.

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