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

ANDREI NICULAE[1,2], CRISTIANA DAVID[1,2], RAZVAN FLORIN ION DRAGOMIRESCU[1,2], ILEANA PERIDE[1,2]*, FLAVIA LILIANA TURCU[1,2], LUCIAN CRISTIAN PETCU[3], ADRIAN COVIC[4,5], IONEL ALEXANDRU CHECHERITA[1,2]

[1] Sf. Ion Emergency Clinical Hospital, Department of Nephrology and Dialysis, 13 Vitan Barzesti Road, 042122, Bucharest, Romania

[2] Carol Davila University of Medicine and Pharmacy Bucharest, Clinical Department No. 3, 37 Dionisie Lupu Str., 020021, Bucharest, Romania

[3] Ovidius University Constanta, Faculty of Dental Medicine, Department of Biostatistics and Biophysics,124 Mamaia Blvd.,900527, Constanta, Romania

[4] Dr. C. I. Parhon Clinical Hospital Iasi Department of Nephrology, Iasi, Romania

[5] Grigore T. Popa University of Medicine and Pharmacy Iasi, Department of Internal Medicine I, 16 Universitatii Str., 700115, Iasi, Romania

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 $Asn^{24,38,83}$) 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,

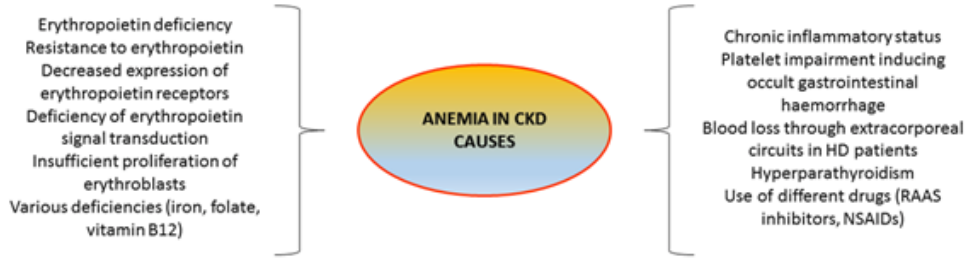


Fig. 1. Different causes of anemia in CKD patients

* email: ileana_peride@yahoo.com; Phone:+40.21.318.07.19

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 benefic influence on hematocrit levels?

This study was aimed to assess the correlation between the presence/ the 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-Erytropoietin (human clone t HEPOFL 13 protein moiety), glycoform beta and its formula is: C809-H1301-N229-O240-S5 [32]. Treatment with the r-HuEPO was conducted according to the guidelines in force, as follows: considering the monthly biochemical tests r-HuEPO dose was maintained when Hb levels were constant, reduced by 25% in case of increased hemoglobin with more than 1 g/dL, increased by 25% in case of hemoglobin decrease with more than 1 g/dL; when hemoglobin increased with more than 2 g/dL we reduced the r-HuEPO dose by 25-50%. R-HuEPO administration was ceased if hemoglobin levels exceeded 13 g/dL for 3 consecutive months, during which the doses were adjusted according to the above mentioned recommendations [33].

Because of the slower absorbtion 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).

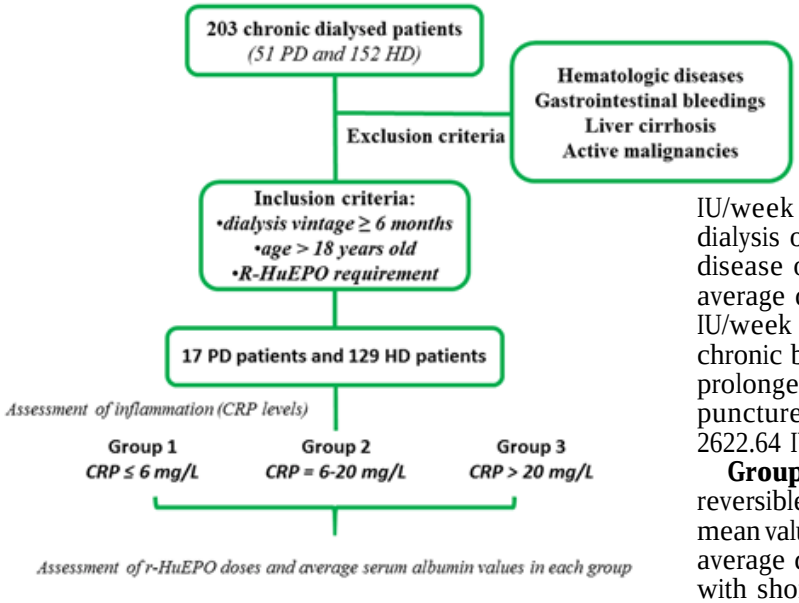


Fig. 2. The study design diagram

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

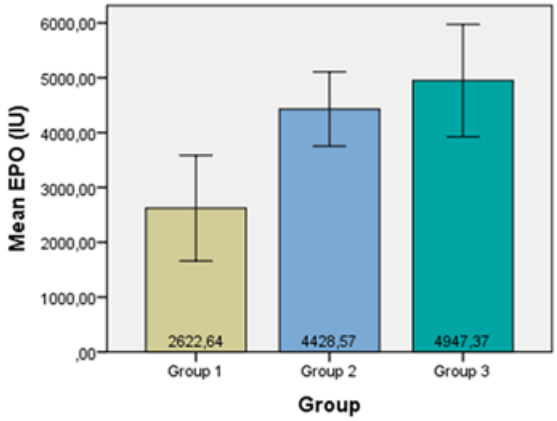
within the group patients with doses up to 6000 IU/week. **The average group r-HuEPO dose was of 4428.57 IU (SD = 676.12 IU).**

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).



Legend: Group 1: CRP < 6 mg/L; Group 2: CRP 6-20 mg/L; Group: CRP > 20 mg/L

Fig. 3. Necessary average r-HuEPO doses – correlated with the degree of inflammation

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 baseline range interval between 12 and 15 U/L, values sufficient enough to maintain an adequate balance between erythrocytes production and apoptosis [25,36-39]. While human experimental studies showed a 10-fold increase of endogenous EPO baseline levels in case of Hb dropped values from 15 to 12 g/dL (post-phlebotomy), when exogenous r-HuEPO is administrated, the following features are noticed (especially after intravenous administration): immediate high serum EPO levels, followed by high peak concentration and marked decrease of its serum values, even to significant low levels, in some cases [25,40,41]. These rapid changes of EPO serum concentration could be responsible to the development of long-term harmful side effects [2,24,25]. Thus, erythropoietin way of administration is just a small piece of the puzzle in achieving the medical purpose for improving the quality of life in dialyzed patients, because chronic inflammation is influenced by several important risk factors. Previously conducted trials including HD patients showed that several factors as increased oxygen species, atherosclerosis, hypoalbuminemia and malnutrition, hyperhomocysteinemia, TNF-α (tumor necrosis factor-alpha), IL-8 and -6 (interleukin) and leptin have a clear contribution in maintaining a continuous inflammatory state [42,43].

As previously mentioned there are still inconsistent and controversial data regarding the optimal correction of anemia in CKD, but the current opinion of Kidney Disease Improving Global Outcomes (KDIGO) guidelines recommend ESA administration to maintain a target Hb value of 11.5g/dL in this category of population (including dialysis patients) [2,44]. In 2013, European Renal Best Practice (ERBP) published its statement, emphasizing the need to maintain an Hb concentration of 10-12 g/dL in general CKD population under ESA administration, and lower Hb values in some special conditions: diabetes mellitus (associating symptomatic limb arteriopathy, stroke, and non-symptomatic ischemic heart disease), malignancies or hypo-responsiveness to ESA therapy [45].

R-HuEPO resistance represents the situation in which, even after the administration of maximum doses of ESA, the recommended level of Hb cannot be obtained, situation noticed in a significant proportion of CKD patients [46]. It is known that there is a correlation between erythropoietin treatment resistance, malnutrition and inflammation in HD and peritoneal dialysis (DP) population [46-48]. Treatment resistance is often associated with several disorders and/or deficiencies: uncontrolled hyperparathyroidism, iron and/or folate deficiency, abuse of aluminium chelators, different hemolytic states, and repeated infection events [49]. Furthermore, according to the literature, the presence of the antibodies or marrow fibrosis as consequences of EPO administration does not correlate with therapy resistance [49].

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%).

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 ($p < 0.001$), with a high proportion of severe malnutrition (21%).

Summarizing, the average doses of r-HuEPO increased together with the CRP values up to 20, after that a saturation effect being achieved. Group 1 and 2 presented statistical significant different average r-HuEPO doses, while group

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 attempts leading only to adverse effects of erythropoietin therapy.

Chronic inflammation is an important condition in maintenance dialysis patients. It affects the EPO necessary and it requires targeted specific treatment, since the increasing EPO doses cannot improve hemoglobin levels when inflammation persists.

References

1.KAZORY, A., ROSS, E.A., J. Am. Coll. Cardiol., **53**, nr. 8, 2009, p. 639
2.LAMMERICH, A., BALCKE, P., BIAS, P., MANGOLD, S., WIESHOLZER, M., Study. Clin. Ther., **38**, nr. 2, 2016, p. 276.e4
3.ESCHBACH, J.W., ADAMSON, J.W., Kidney Int., **28**, nr. 1, 1985, p. 1
4.KAZMI, W.H., KAUSZ, A.T., KHAN, S., ABICHANDANI, R., RUTHAZER, R., OBRADOR, G.T., PEREIRA, B.J., Am. J. Kidney Dis., **38**, nr. 4, 2001, p. 803
5.ASTOR, B.C., MUNTNER, P., LEVIN, A., EUSTACE, JA, CORESH, J., Arch. Intern. Med., **162**, nr. 12, 2002, p. 1401
6.LEVEY, A.S., CORESH, J., Lancet, **739**, nr. 9811, 2012, p. 165
7.DEL VECCHIO, L., LOCATELLI, F., Expert Rev. Hematol., **7**, nr. 4, 2014, p. 495
8.MCCLELLAN, W., ARONOFF, S.L., BOLTON, W.K., HOOD, S., LORBER, D.L., TANG, K.L., TSE, T.F., WASSERMAN, B., LEISEROWITZ, M., Curr. Med. Res. Opin., **20**, nr. 9, 2004, p. 1501
9.SINGH, A.K., SZCZECH, L., TANG, K.L., BARNHART, H., SAPP, S., WOLFSON, M., REDDAN, D.; CHOIR INVESTIGATORS, N. Engl. J. Med., **355**, nr. 20, 2006, p. 2085
10.BESARAB, A., BOLTON, W.K., BROWNE, J.K., EGRIE, J.C., NISSENSON, A.R., OKAMOTO, D.M., SCHWAB, S.J., GOODKIN, D.A., N. Engl. J. Med., **339**, nr. 9, 1998, p. 584
11.AL-AHMAD, A., RAND, W.M., MANJUNATH, G., KONSTAM, M.A., SALEM, D.N., LEVEY, A.S., SARNAK, M.J., J. Am. Coll. Cardiol., **38**, nr. 4, 2001, p. 955
12.MCCLELLAN, W.M., FLANDERS, W.D., LANGSTON, R.D., JURKOVITZ, C., PRESLEY, R., J. Am. Soc. Nephrol., **13**, nr. 7, 2002, p. 1928
13.COLLINS, A.J., Adv. Stud. Med., **3**, 2003, p. S194
14.PFEFFER, M.A., BURDMANN, E.A., CHEN, C.Y., COOPER, M.E., DE ZEEUW, D., ECKARDT, K.U., FEYZI, J.M., IVANOVICH, P., KEWALRAMANI, R., LEVEY, A.S., LEWIS, E.F., MCGILL, J.B., MCMURRAY, J.J., PARFREY, P., PARVING, H.H., REMUZZI, G., SINGH, A.K., SOLOMON, S.D., TOTO, R.; TREAT INVESTIGATORS, N. Engl. J. Med., **361**, nr. 21, 2009, p. 2019
15.LOCATELLI, F., PISONI, R.L., COMBE, C., BOMMER, J., ANDREUCCI, V.E., PIERA, L., GREENWOOD, R., FELDMAN, H.I., PORT, F.K., HELD, P.J., Nephrol. Dial. Transplant., **19**, nr. 1, 2004, p. 121 [Erratum, Nephrol. Dial. Transplant., **19**, nr. 6, 2004, p. 1666]
16.FOLEY, R.N., PARFREY, P.S., HARNETT, J.D., KENT, G.M., MURRAY, D.C., BARRE, P.E., Am. J. Kidney Dis., **28**, nr. 1, 1996, p. 53
17.KEANE, W.F., BRENNER, B.M., DE ZEEUW, D., GRUNFELD, J.P., MCGILL, J., MITCH, W.E., RIBEIRO, A.B., SHAHINFAR, S., SIMPSON, R.L., SNAPINN, S.M., TOTO, R.; RENAAL STUDY INVESTIGATORS, Kidney Int., **63**, nr. 4, 2003, p. 1499
18.VLAGOPOULOS, P.T., TIGHIOUART, H., WEINER, D.E., GRIFFITH, J., PETTITT, D., SALEM, D.N., LEVEY, A.S., SARNAK, M.J., J. Am. Soc. Nephrol., **16**, nr. 11, 2005, p. 3403
19.TONG, P.C.Y., KONG, A.P.S., SO, W.Y., NG, M.H., YANG, X., NG, M.C., MA, R.C., HO, C.S., LAM, C.W., CHOW, C.C., COCKRAM, C.S., CHAN, J.C., Diabetes Care, **29**, nr. 11, 2006, p. 2439
20.OBERADOR, G.T., PEREIRA, B.J., Nephrol. Dial. Transplant., **17**, Suppl 11, 2002, p. 44
21.JELKMANN, W., Physiology and pharmacology of erythropoietin. Transfus. Med. Hemother., **40**, nr. 5, 2013, p. 302
22.NOWROUSIAN, M.R., Springer, Wien, 2002, p. 203
23.JELKMAN, W., Br. J. Haematol., **141**, nr. 3, 2008, p. 287
24.UNGER, E.F., THOMPSON, A.M., BLANK, M.J., TEMPLE, R., N. Engl. J. Med., **362**, nr. 3, 2010, p. 189
25.FISHBANE, S., BESARAB, A., Clin. J. Am. Soc. Nephrol., **2**, nr. 6, 2007, p. 1274
26.PROVATOPOULOU, S.T., ZIROYIANNIS, P.N., Hippokratia, **15**, nr. 2, 2011, p. 109
27.DAVID, C., PERIDE, I., NICULAE, A., CONSTANTIN, A.M., CHECHERITA, I.A., BMC Nephrol., **17**, nr. 1, 2016, p. 131
28.CHECHERITA, I.A., DAVID, C., STOICA, L., POPESCU, P., CIOCALTEU, A., LASCAR, I., Rom. J. Morphol. Embryol., **52**, nr. 2, 2011, p. 533
29.CHECHERITA, I.A., DAVID, C., CIOCALTEU, A., LASCAR, I., BUDALA, L., Rom. J. Morphol. Embryol., **54**, nr. 3, 2013, p. 539
30.CHECHERITA, I.A., DAVID, C., DIACONU, V., CIOCALTEU, A., LASCAR, I., Rom. J. Morphol. Embryol., **52**, Suppl 3, 2011, p. 1047
31.CHECHERITA, I.A., MANDA, G., HINESCU, M.E., PERIDE, I., NICULAE, A., BILHA, S., GRAMATICU, A., VORONEANU, L., Covic, A., Int. Urol. Nephrol., **48**, nr. 3, 2016, p. 373
32.*** https://www.drugs.com/international/epoetin-beta.html
33.*** http://legislatie.just.ro/Public/DetaliiDocument/129068
34.JELKMANN, W., Transfus. Med. Hemother., **40**, nr. 5, 2013, p. 302
35.ZACHEE, P., Drugs, **49**, nr. 4, 1995, p. 536
36.KWAAN, H.C., WANG, J., Semin. Thromb. Haemost., **29**, nr. 5, 2003, p. 451
37.MOVILLI, E., PERTICA, N., CAMERINI, C., CANCARINI, G.C., BRUNORI, G., SCOLARI, F., MAIORCA, R., Am. J. Kidney Dis., **39**, nr. 4. 2000, p. 850
38.SARAN, R., BRAGG-GRESHAM, J.L., RAYNER, H.C., GOODKIN, D.A., KEEN, M.L., VAN DIJK, P.C., KUROKAWA, K., PIERA, L., SAITO, A., FUKUHARA, S., YOUNG, E.W., HELD, P.J., PORT, F.K., Kidney Int., **64**, nr. 1, 2003, p. 254
39.SUNDAL, E., KAESER, U., Nephrol. Dial. Transplant., **4**, nr. 11, 1989, p. 979
40.MAEDA, H., HITOMI, Y., HIRATA, R., TOHYAMA, H., SUWATA, J., KAMATA, S., FUJINO, Y., MURATA, N., Int. J. Hematol., **55**, nr. 2, 1992, p. 111
41.ERSLEV, A.J., N. Engl. J. Med., **324**, nr. 19, 1991, p. 1339
42.KALANTAR-ZADEH, K., Semin. Dial., **18**, nr. 5, 2005, p. 365
43.SILVERSTEIN, D.M., Pediatr. Nephrol., **24**, nr. 8, 2009, p. 1445
44.*** Kidney Disease Improving Global Outcomes. Kidney Int. Suppl., **2**, nr. 4, 2012, p. 279
45.LOCATELLI, F., BÁRÁNY, P., COVIC, A., DE FRANCISCO, A., DEL VECCHIO, L., GOLDSMITH, D., HORL, W., LONDON, G., VANHOLDER, R., VAN BIESEN, W.; ERA-EDTA ERBP ADVISORY BOARD, Nephrol. Dial. Transplant., **28**, nr. 6, 2013, p. 1346
46.DEL VECCHIO, L., POZZONI, P., ANDRULLI, S., LOCATELLI, F., J. Ren. Nutr., **15**, nr. 1, 2005, p. 137
47.AFSAR, B., Int. J. Artif. Organs., **36**, nr. 5, 2013, p. 314
48.PÉREZ-FLORES, I., CORONEL, F., CIGARRÁN, S., HERRERO, J.A., CALVO, N., Adv. Perit. Dial., **23**, 2007, p. 140
49.DRÜEKE, T.B., Am. J. Nephrol., **10**, Suppl 2, 1990, p. 34
50.STEVINKEL, P., KETTELER, M., JOHNSON, R.J., LINDHOLM, B., PECOITS-FILHO, R., RIELLA, M., HEIMBÜRGER, O., CEDERHOLM, T., GIRNDT, M., Kidney Int., **67**, nr. 4, 2005, p. 1216
51.GIRNDT, M., KAUL, H., LEITNAKER, C.K., SESTER, M., SESTER, U., KÖHLER, H., Am. J. Kidney Dis., **37**, nr. 5, 2001, p. 954
52.ISVORANU, I., RADULESCU, D., PERIDE, I., NICULAE, A., SINESCU, R.D., CHECHERITA, I.A., Rev. Chim. (Bucharest), **66**, no. 8, 2015, p. 1239
53.ISVORANU, I., PERIDE, I., RADULESCU, D., NICULAE, A., SINESCU, R.D., CHECHERITA, I.A., Rev. Chim. (Bucharest), **66**, no. 9, 2015, p. 1316
54.KALANTAR-ZADEH, K., Semin. Dial., **18**, no. 5, 2005, p. 365

Manuscript received: 19.12.2016