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ERYTHROPOIETIN PRODUCTION IN PATIENTS WITH MALIGNANT LYMPHOMA

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A b s t r a c t: Lymphomas are a heterogenous group of malignant diseases. 30–50% of patients, even before chemo- and radiotherapy are begun have anemia. The pathogenesis of this anemia is multifactorial and still not completely clear. Newer investigations refer to a causality between the anemia in patients with lymphoma and the inappropriate erythropoietin production for the degree of anemia. Based on this finding, the aim of the study was to evaluate the erythropoietin production in patients with malignant lymphoma in order to define the clinical conditions of Epo deficiency and thereby enable rational use of this expensive drug.

27 patients with malignant lymphoma were examined. The control group consisted of 25 patients with iron deficiency anemia. 14 healthy volunteers represented the so-called "normal" control. The adequacy of Epo production was estimated from the graphic representation of the linear regression between Epo and hemoglobin (Hb) in the control group, as well as from the O/PEpo ratio as a measure of the degree of adequacy of Epo production (O – observed Epo value, P-predicted Epo value from the regression equation of the control group). The erythropoietic activity was estimated from the graphic representation of the linear regression between soluble transferin receptors (sTfR) and Hb in the control group, as well as from the O/PsTfR ratio, as a measure of the degree of adequacy of erythropoietic activity (O – observed sTfR value, P – predicted sTfR value from the regression equation of the control group).

Significant inverse correlation between Epo and Hb was found in patients with malignant lymphoma ($r = –0.76$, $p < 0.001$). 33% of patients had inadequate Epo response to anemia. O/PEpo in patients with malignant lymphoma is significantly lower in comparison to the control group, which also points to the inadequacy of erythropoietin production. There was a significant negative correlation between sTfR and Hb in patients with malignant lymphoma ($r = –0.056$, $p < 0.001$). Inadequate sTfR response to anemia have 76% of patients. The positive correlation between O/PEpo and O/PsTfR ($r = 0.79$, $p < 0.001$) points out to a causality between the inadequacy of erythropoietin production and the inadequate erythropoiesis.

In conclusion, results from this study show unambiguously that anemia in patients with malignant lymphoma appears because of decreased erythropoiesis as a consequence of bone marrow infiltration with lymphoma cells as well as inadequate Epo production. Most probably, the inadequate Epo production in patients with malignant lymphoma is as that seen in the anemia of chronic diseases whose mechanism is not clear.

Key words: malignant lymphoma, erythropoietin, soluble transferin receptors.

Introduction

Lymphomas are a heterogeneous group of malignant disorders which show significant variations in their clinical course and outcome. 30–50% of patients, depending on the pathohistological subtype, even before chemo- and/or radiotherapy are begun, have anemia [1]. The pathogenesis of this anemia is multifactorial and still not completely clear. Bone marrow infiltration and autoimmune haemolysis are among the most often mentioned possible causes. Newer investigations refer to a causality between the anemia in patients with lymphoma and inappropriate erythropoietin production for the degree of anemia [2, 3, 4].

Also, therapy with recombinant human erythropoietin (rHuEpo) shows that inappropriate erythropoietin production should not be neglected as a factor in the pathogenesis of anemia in patients with malignant lymphoma [5, 6, 7].

Based on these findings, the aim of the study was to evaluate the erythropoietin production in patients with malignant lymphoma in order to define the clinical conditions of Epo deficiency and thereby the group of patients which would mostly benefit from therapy with rHuEpo.

Patients and methods

27 patients with malignant lymphoma were examined. Their disease stadium was defined according to the Ann Arbor "staging" system [8]. The patients received the following chemotherapy: MOPP or ABVD and CHOP or CVP for Hodgkin, respectively non-Hodgkin lymphoma. All routine laboratory parameters were determined for each of the patients. The following were separately analyzed for the purpose of excluding iron deficiency as well as renal anemia: Hb, Ht, Er and erythrocyte indexes, erythrocyte morphology, serum iron, TIBC (total iron binding capacity), feritin, and creatinin.

The control group consisted of 25 patients with iron deficiency anemia. Blood loss, through excessive menstrual bleeding or a bleeding gastric/duodenal ulcer was the reason for the anemia. As the erythropoietin response in iron deficiency anemia is known and considered to be appropriate, iron deficiency anemia patients were chosen for a control group [9].

14 healthy volunteers represented the so-called "normal" control.

On principle the assessment of adequacy of erythropoietin production is based on a mathematical comparison of serum Epo concentration in patients with malignant lymphoma and referent subjects with similar hemoglobin value. In our study the adequacy of Epo production was estimated from the graphic representation of the linear regression between Epo and hemoglobin (Hb) in the control group, as well as from the O/PEpo ratio as a measure of the degree of adequacy of Epo production (O-observed Epo value, P-predicted Epo value from the regression equation of the control group: logEpo = 3.925–0.234Hb). Erythropoietin production is considered inappropriate if O/P < 0.8, as determined from the 95% confidence interval for O/PEpo in the control group which amounts: 0.80–1.2.

Soluble transferin receptors (sTfR) were used to assess the erythropoietic activity according to the well-known link between the concentration of sTfR and erythron activity. The erythropoietic activity was assessed from the graphic representation of the linear regression between soluble transferin receptors (sTfR) and Hb in the control group, as well as from the O/P ratio, as a measure of the degree of adequacy of erythropoietic activity (O-observed sTfR value, P-predicted sTfR value from the regression equation of the control group: logsTfR = 4.526–0.029Hb). Yet the erythropoietic activity is considered inappropriate if O/PsTfR < 0.9, as determined from the 95% confidence interval for the O/PsTfR in the control group, which amounts 0.9–1.1.

Serum erythropoietin and the solubile transferin receptors were measured with a commercial RIA, respectively ELISA assay.

In the statistical analysis, the values for Epo and sTfR were transformed into logarithmic ones. The following statistical tests were used: Student t-test, ANOVA, Pearson's test, linear regression analysis, analysis of covariance.

Results

The characteristics of patients with malignant lymphoma are given in Table 1. Of the total of 27 patients (mean age 57 ± 13 years), 18 had non-Hodgkin and 9 had Hodgkin lymphoma. According to the Ann Arbor classification 4 patients were in the second, 8 in the third and 15 in the fourth disease stadium. 12 patients were pretreated with chemotherapy. The anemia was moderate in most of the patients (mean Hb 8.9 ± 1.1 g/dl) and became severe within the advanced disease stadium (range 6.7–10.1 g/dl). Serum iron, ferritin and creatinin show that iron deficiency or renal failure did not cause the anemia. In patients with malignant lymphoma, serum Epo values were significantly higher than in healthy volunteers (57.3 ± 33.9 *v* 13.9 ± 3.8mU/ml, p < 0.05) (fig. 1). Serum Epo was not significantly different either between patients from different

disease stadiums or between chemotherapy pretreated and not pretreated patients.

Table 1 – Табела 1

Characteristics of patients with malignant lymphoma
Карактеристики на пациентите со малигген лимфом

Patients (n=27)	mean value±SD
non-Hodgkin/Hodgkin lymphoma	18 / 9
Age (range)	57 ± 13 (39–68)
Sex (m/f)	17 / 10
Disease stadium I/ II/ III/ IV	0/ 4/ 8/ 15
Chemotherapy pretreated Yes/No	12/15
Hb (g/dl) (range)	8.9 ± 1.1 (6.7–10.3)
Serum iron (μmol/l)	17.3 ± 2.5
Ferritin (μg/l)	218 ± 38.2
Creatinin (mmol/l)	78 ± 12.5

There is a significant inverse correlation between Epo and Hb in patients with malignant lymphoma: $r = -0.76$, $p < 0.001$. Erythropoietin production was inappropriate in 9 patients (33%) in whom serum Epo values were below the 95% confidence interval of the control group (fig. 2).

The adequacy of Epo response to anemia has been also estimated through determination of the O/PEpo ratio, which in the advanced disease stadiums appeared to be significantly lower compared to the controls (Table 2).

sTfR values are shown separately for each patient with malignant lymphoma and healthy individuals (Fig. 3). In patients with malignant lymphoma sTfR values are significantly higher (9031 ± 3513 *v* 5470 ± 1670ng/ml, $p<0.05$). Treatment of patients did not interfere with sTfR values. A significant negative correlation between sTfR and Hb was found: $r = -0.056$, $p < 0.001$ (fig. 4).

13 patients with malignant lymphoma had inappropriate erythropoietic activity because their values for sTfR were below the 95% confidence interval of the control group. Most of these patients were in the third, or the fourth disease stadium. To estimate causality between the inappropriate erythropoietin production and erythropoiesis we looked for a correlation between O/PEpo and O/PsTfR (fig. 5). A positive correlation between O/PEpo and O/PsTfR ($r = 0.79$, $p < 0.001$) was found which points to an Epo-driven erythropoiesis. In the figure the rectangle indicates the 95% confidence interval and the horizontal and vertical lines indicate the lower limit of the O/PEpo, respectively O/PsTfR value.

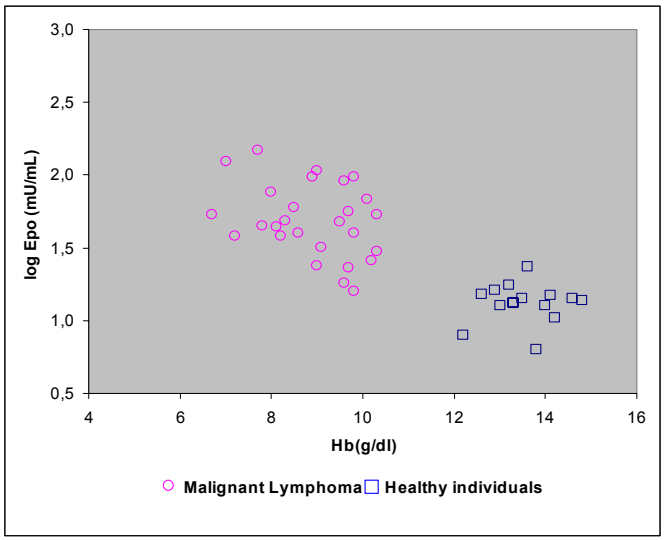

Figure 1 – ***Epo*** *in patients with malignant lymphoma and healthy individuals*

Слика 1 – ***Epo*** *кај пациенти со малигген лимфом и здрави испитаници*

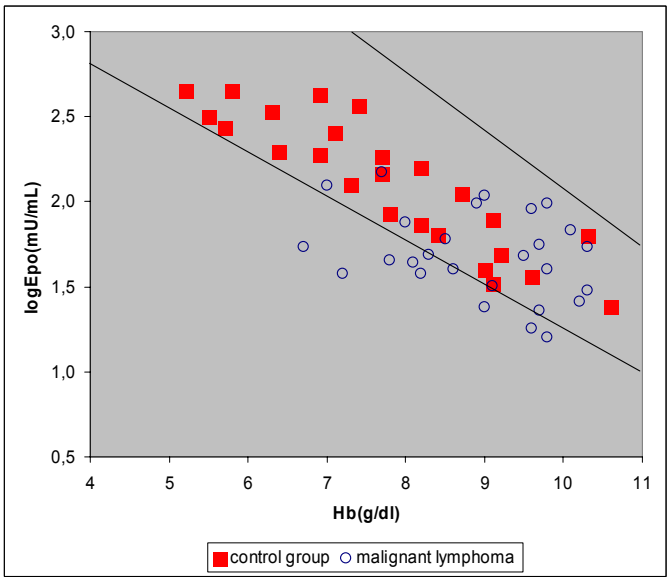

Figure 2 – *Correlation between erythropoietin and hemoglobin in patients with malignant lymphoma*

Слика 2 – *Корелација меѓу еритропоетинот и хемоглобинот кај пациенти со малигген лимфом*

Table 2 – Табела 2

O/P Epo *in patients with malignanat lymphoma*

O/P Epo *кај пациенти со малигген лимфом*

	O/P Epo
Patients with malignant lymphoma (n = 27) I stadium (n = 0) II stadium (n = 4) III stadium (n = 8) IV stadium (n=15)	 / 0.86 ± 0.14 0.81 ± 0.02 0.70 ± 0.06
Control	1.00 ± 0.11

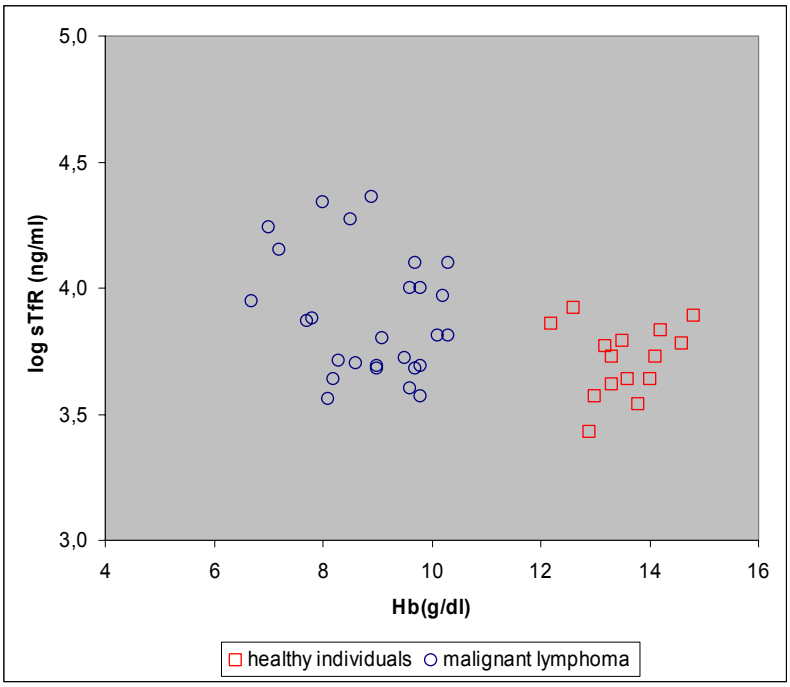

Figure 3 – ***sTfR*** *in patients with malignant lymphoma and healthy individuals*

Слика 3 – ***sTfR*** *кај пациенти со малигген лимфом*

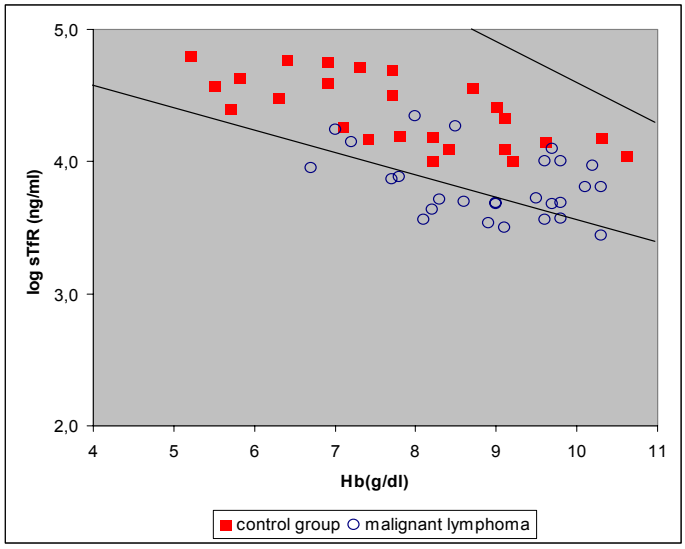

Figure 4 – *Correlation between* ***sTfR*** *and* ***Hb*** *in patients with malignant lymphoma*

Слика 4 – *Корелација меѓу* ***sTfR*** *и* ***Hb*** *кај пациенти со малигген лимфом*

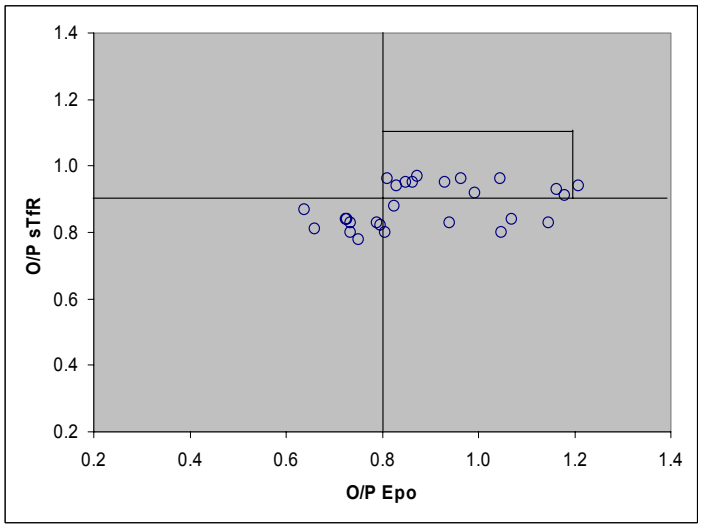

Figure 5 – *Correlation between* ***O/P Epo*** *and* ***O/P sTfR*** *in patients with malignant lymphoma*

Слика 5 – *Корелација меѓу* ***O/P Epo*** *и* ***O/P sTfR*** *кај пациенти со малигген лимфом*

Discussion and conclusions

Our results show that in 48% of patients with malignant lymphoma the erythropoietic activity is reduced in accordance with the sTfR values. Most of them were patients in the third or the fourth disease stadium. On the other hand, 33% of the patients with malignant lymphoma had inappropriate Epo production for the degree of anemia. Low values for O/PEpo were found particularly in patients in the fourth disease stadium. Patients with inappropriate Epo also had an inappropriate sTfR response to anemia which is confirmed by the correlation between O/PEpo and O/PsTfR.

Reduced Epo production in patients with malignant lymphoma has also been found by others [7, 10]. From the relatively small number of studies which evaluate the efficacy of recombinant erythropoietin for the treatment of anemia in patients with malignant lymphoma it was realized that the efficacy of rHuEpo is greater in patients with inappropriate Epo production. Patients with high serum Epo values needed much higher doses of recombinant erythropoietin to achieve a reasonable therapeutic effect [10, 11, 12].

In general, a lot of studies which assess the pathogenesis of anemia in patients with malignant diseases show that, in these patients, the anemia does not stimulate an appropriate Epo response [13, 14, 15, 16, 17, 18, 19, 20, 21]. In most of the cases patients do not show an appropriate increase in erythropoietin concentration with decreasing hemoglobin concentration as is the case with iron deficiency anemia.

Inhibition of Epo production is thought to be linked to many cytokines (Il-1, TNF, TGF-β, interferons) which otherwise take part in the initiation and mediation of the inflammatory and immunological reactions in the human body [22]. Increased serum concentrations of Il-1, TNF, INF-γ, TGF-β, Il-6 are often found in patients with different cancers [23, 24, 25, 26, 27, 28]. Il-1, TNF and TGF in experiments with human hepatoma cell line (model system for investigation of Epo production regulation) inhibit the Epo production under hypoxic conditions. On the contrary, Il-6 stimulates it [29]. The inhibitory action of these cytokines on the erythroid precursors has been antagonized by supplementing high Epo doses [30].

In conclusion, the reduced Epo production has an important role in the pathogenesis of anemia in a significant number of patients with malignant lymphoma. Unambigously, the anemia in patients with malignant lymphoma appears because of the decreased erythropoiesis as a consequence of bone marrow infiltration with lymphoma cells as well as inadequate Epo production. Most probably, the inadequate Epo production in patients with malignant lymphoma is as that seen in the anemia of chronic diseases whose mechanism is not clear. Therefore the indications for treatment with rHuEpo should be based on

the adequacy of endogenous Epo to the degree of anemia, taking into consideration the fact that the concentration of endogenous erythropoietin is not the only parameter of therapy response to rHuEpo.

REFERENCES

1. Moullet J., Salles G., Ketterer N. *et al*. (1998): Frequency and significance of anemia in non-Hodgkin's lymphoma patients. *Ann Oncol*. 9: 1109–1115.

2. Salles G., Bienvenu J., Barbier Y. *et al*. (1996): Elevated levels of alfa-TNF and p55-R-TNF in lymphoma patients and their prognostic significance. *Br J Haematol.* 93: 352–359.

3. Boyd H. K., Lappin T. R. J. (1991): Erythropoietin deficiency in the anemia of chronic disorders. *Eur J Haematol.* 45: 198–201.

4. Johnson C. S., Cook C. A., Furmanski P. (1990): In vivo suppression of erythropoiesis by tumor necrosis factor alfa (TNF-alfa): reversal with exogenous erythropoietin (EPO). *Exp Hematol.* 18 (2): 109–113.

5. Cazzola M., Messinger D., Battistel V. *et al*. (1995): Recombinant human erythropoietin in the anemia associated with multiple myeloma or non-Hodgkin's lymphoma: dose finding and identification of the predictors of response. *Blood.* 86: 4446–4453.

6. Osterborg A., Boogaerts M. A., Cimino R. *et al*. (1996): Recombinant human erythropoietin in transfusion-dependent anemic patients with multiple myeloma and non-Hodgkin's lymphoma – a randomised multicentre study. *Blood.* 87: 2675–2682.

7. Osterborg A., Brandberg Y., Molostova V. *et al*. (2002): Randomised, double-blind, placebo-controlled trial of recombinant human erythropoietin, epoetin beta, in hematologic malignancies. *J Clin Oncol.* 20: 2486–2494.

8. Carbone P. P., Kaplan H. S., Mushoff K. *et al*. (1971): Report of the committee on Hodgkin's disease staging classification. *Cancer Res.* 311: 860.

9. Barosi G., Cazzola M., Stefanelli M. (1985): Classification of anemia by means of ferrokinetic parameters. *Br J Haematol.* 61: 357–370.

10. Ariad S., Clifford D., Penfold G. (1992): Erythropoietin response in anaemic patients with multiple myeloma and other lymphoid malignancies infiltrating the bone marrow. *Eur J Haematol.* 49: 59–62.

11. Oster W., Friedhelm H., Heinhold G. *et al*. (1990): Erythropoietin for the treatment of anemia of malignancy associated with neoplastic bone marrow infiltration. *J Clin Oncol.* 8: 956–962.

12. Platanias L. C., Miller C. B., Mick R. *et al*. (1991): Treatment of chemotherapy-induced anemia with recombinant human erythropoietin in cancer patients. *J Clin Oncol.* 9: 2021–2026.

13. Cox R., Musial T., Gyde O. H. B. (1986): Reduced erythropoietin levels as a cause of anemia in patients with lung cancer. *Eur J Cancer Clin. Oncol* 22: 511–514.

14. Dainiak N., Kulkarni V., Howard D. *et al*. (1983): Mechanism of abnormal erythropoietin in malignancy. *Cancer*. 57: 1101–1106.

15. Barlogie B., Beck T. (1993): Recombinant human erythropoietin and the anemia of multiple myeloma. *Stem Cells.* 11: 88–94.

16. Ludwig H., Fritz E., Kotzmann H., Gisslinger H. (1990): Erythropoietin treatment of anemia associated with multiple myeloma. *New Engl J Med.* 322: 1693–1699.

17. Boyd H. K., Lappin T. R. J. (1991): Erythropoietin deficiency in the anemia of chronic disorders. *Eur J Haemotol.* 46: 198–201.

18. Miller C. B., Jones R. J., Piantadosi S. *et al*. (1990): Decreased erythropoietin response in patients with the anemia of cancer. *New Engl J Med.* 322: 1689–1692.

19. Ward H. P., Kurnick J. E., Pisarezyk M. J. (1971): Serum level of erythropoietin in anemias associated with chronic infection, malignancy and primary hematopoietic disease. *J Clin Invest.* 50: 332–335.

20. Mittelman M. (1996): Anemia of cancer: Pathogenesis and treatment with recombinant human erythropoietin-an improved quality of life. *Isr J Med Sci.* 32: 1201–1206.

21. Mittelman M., Zeidman A., Fradin Z. *et al*. (1997): Recombinant Human Erythropoietin in the Treatment of Multipe Myeloma-Associated Anemia. *Acta Haematol.* 98: 204–210.

22. Cartwright G. E. (1966): The anemia of chronic disorders. *Sem Hematol.* 3: 351–375.

23. Jelkmann W., Fandrey J. (1993): Role of inflammatory cytokines in the pathophysiology of erythropoietin response. *Erythropoiesis.* 4: 10–15.

24. Srinivasiah J., Jacobs P. C., Rozmiarek S. K. *et al*. (1993): Studies in nonhuman primates on the pathogenesis of rh-IL-6 related anemia. *ProcAmSocClinOncol.* 86: 2733–2736.

25. Naumovski J., Utz P. J., Bergstro S. K. (1989): SUP-HDI: a new Hodgkin's disease derived cell line with lymphoid features produces interferon-gamma. *Blood.* 74: 2733–2742.

26. Kremer J. P., Reisbach G., Neri C. *et al*. (1992): B-cell chronic lymphocytic leukemia cells express and release transforming growth factor-beta. *BrJHaematol.* 80: 480–487.

27. Emillie D., Coumbaras J., Raphael M. *et al*. (1992): Interleukin-6 production in high-grade B lymphoma: correlation with the presence of malignant immunoblasts in acquired immunodeficiency syndrome and in human immunodeficiency virus-seronegative patients. *Blood.* 80: 498–504.

28. Doweiko J. P., Goldberg M. A. (1991): Erythropoietin therapy in cancer patients. *Oncology.* 5: 31–37.

29. Faquin W. C., Schenider T. J., Goldberg M. A. (1992): Effect of inflammatory cytokines on hypoxia-induced erythropoietin production *Blood.* 79: 1987–1994.

30. Means R. T., Krantz S. B. (1991): Inhibition of human erythroid colony-forming units by gamma interferon can be corrected by recombinant human erythropoietin. *Blood.* 78: 2564–2567.

Резиме

ЕРИТРОПОЕТИНСКА ПРОДУКЦИЈА КАЈ ПАЦИЕНТИ СО МАЛИГЕН ЛИМФОМ

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Апстракт: Лимфомите се хетерогена група малигноми. 30–50% од пациентите, уште пред почнување со хемо- и радиотерапијата имаат анемија. Патогенезата на анемијата е мултифакториелна и сè уште не е наполно јасна. Понови испитувања укажуваат на поврзаност на анемијата кај пациентите со лимфоми и несоодветната продукција на еритропоетин за даден степен анемија. Од овие сознанија, произлезе целта на испитувањето: да се евалуира еритропоетинската продукција кај пациентите со малиген лимфом како би се дефинирале клиничките состојби пратени со недостиг на **Epo** и групата пациенти кои би имале најголема корист од терапијата со **rHuEpo**.

Испитувани се 27 пациенти со малиген лимфом. Контролната група ја сочинуваат 25 пациенти со анемија од недостиг на железо. 14 здрави испитаници ја претставуваат т.н. „нормална контрола“. Соодветноста на **Epo** продукцијата е проценувана од графичкиот приказ на линеарната регресија меѓу **Epo** и хемоглобинот (**Hb**) кај контролната група, како и врз основа на соодносот **O/PEpo**, како мерка за степенот на соодветноста на **Epo** продукцијата (**O** – опсервирана **Epo** вредност, **P** – пресметана **Epo** вредност од регресионата равенка на контролата). Еритропоетската активност е проценувана од графичкиот приказ на линеарната регресија меѓу солубилните трансфе-

ински рецептори (**sTfR**) и **Hb** кај контролната група и соодносот **O/PsTfR,** како мерка за степенот на соодветноста на еритропоетската активност (**O** – опсервирана **sTfR** вредност, **P** – пресметана **sTfR** вредност од регресионата равенка на контролата).

Најдена е сигнификантна инверзна корелација меѓу **Epo** и **Hb** кај пациентите со малиген лимфом (**r** = –0.76, **p** < 0.001). 33% од пациентите имаат несоодветен **Epo** одговор од анемијата. Вредноста на **O/PEpo** е сигнификантно помала во споредба со контролата и исто така го потврдува постоењето на несоодветна еритропоетинска продукција. Кај пациентите со малиген лимфом постои сигнификантна негативна корелација меѓу **sTfR** и **Hb** (**r** = –0.056, **p** < 0.001). Несоодветен **sTfR** од анемијата имаат 76% од пациентите. Позитивната корелација меѓу **O/PEpo** и **O/PsTfR** упатува на поврзаност меѓу несоодветната еритропоетинска продукција и несоодветната еритропоеза (**r** = 0.79, **p** < 0.001).

Во заклучок може да се каже дека резултатите од оваа студија недвосмислено покажуваат дека анемијата кај пациентите со малиген лимфом се должи на смалената еритропоеза, која е последица на лимфомската инфилтрација на коскената срцевина и на несоодветната **Epo** продукција. При тоа, несоодветната **Epo** продукција кај пациентите со малиген лимфом, најверојатно е од видот на онаа што се среќава кај пациентите со анемија од хронични заболувања и има нејасен механизам на настанување.

Клучни зборови: малиген лимфом, еритропоетин, солубилни трансферински рецептори.