ERYTHROPOIETIN PRODUCTION IN PATIENTS WITH MALIGNANT LYMPHOMA

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Abstract: Lymphomas are a heterogeneous 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 chemotherapy 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 soluble 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 – Таблица 1

<table>
<thead>
<tr>
<th>Characteristics of patients with malignant lymphoma</th>
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<tbody>
<tr>
<td>Karakteristiki na pacientite so maligen limfom</td>
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<table>
<thead>
<tr>
<th>Patients (n=27)</th>
<th>mean value±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>non-Hodgkin/Hodgkin lymphoma</td>
<td>18 / 9</td>
</tr>
<tr>
<td>Age (range)</td>
<td>57 ± 13 (39–68)</td>
</tr>
<tr>
<td>Sex (m/f)</td>
<td>17 / 10</td>
</tr>
<tr>
<td>Disease stadium I/ II/ III/ IV</td>
<td>0/ 4/ 8/ 15</td>
</tr>
<tr>
<td>Chemotherapy pretreated Yes/No</td>
<td>12/15</td>
</tr>
<tr>
<td>Hb (g/dl) (range)</td>
<td>8.9 ± 1.1 (6.7–10.3)</td>
</tr>
<tr>
<td>Serum iron (µmol/l)</td>
<td>17.3 ± 2.5</td>
</tr>
<tr>
<td>Ferritin (µg/l)</td>
<td>218 ± 38.2</td>
</tr>
<tr>
<td>Creatinin (mmol/l)</td>
<td>78 ± 12.5</td>
</tr>
</tbody>
</table>

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 vs 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 малиден лимфом и здрави испитаници

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

Слика 2 – Корелација меѓу еритроийотин и хемогоблінот кај пациенти со малиден лимфом

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Table 2 – Таблица 2

**O/P Epo in patients with malignant lymphoma**

**O/P Епо кај пациенти со малигн лимфом**

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

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

Слика 3 – sTfR кај пациенти со малигн лимфом
Figure 4 – Correlation between sTfR and Hb in patients with malignant lymphoma

Слика 4 – Корелација међу sTfR и Hb кај иациенийи со малигн лимфом

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

Слика 5 – Корелација међу O/P Epo и O/P sTfR кај иациенийи со малигн лимфом
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 (II-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 II-1, TNF, INF-γ, TGF-β, II-6 are often found in patients with different cancers [23, 24, 25, 26, 27, 28]. II-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, II-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. Unambiguously, 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


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 уште не е наполнно јасна. Понови испитувања укажуваат на поврзаност на анемијата кај пациентите со лимфоми и несоодветната продукција на еритропоетин за даден степен анемија. Од овие сознанија, произлегле целта на испитувањето: да се евалуира еритропоетинската продукција кај пациентите со малиген лимфом како би се дефинирале клиничките сгомени 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 контролната). Еритропоетинската активност е проценувана од графичкиот приказ на линеарната регресија меѓу солубилните трансфе-

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рински рецептори (sTfR) и Hb киј 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 сигификантна инверзна корелација меѓу Epo и Hb киј пациентите со малиген лимфом (r = −0.76, p < 0.001). 33% од пациентите имаат несоодветен Epo одговор од анемијата. Вредноста на O/PEpo е сигификантно помала во споредба со контролата и исто така го потврдува постоенето на несоодветна еритропоетичка продукција. Каде пациентите со малиген лимфом постои сигификантна 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 еритропоетичка продукција и несоодветната еритроелоеза (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 смаалената еритроелоеза, која е последица на лимфомската инфилтрирања 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 срчава кај пациентите со анемија од хронични заболувања и има нејасен механизам на настанување.

Ключни зборови: малиген лимфом, еритропоетин, солубилин трансферински рецептори.