The effect of recombinant human erythropoietin on the treatment of anemia of prematurity

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Abstract

Objective: to assess the efficacy of erythropoietin in the prevention and treatment of anemia of prematurity, correlating the use of this drug with weight gain, length, and head circumference and comparing two administration schemes of the same weekly dose: daily use and twice a week.

Methods: the study comprised 42 premature newborns with gestational age up to 33 weeks, birthweight up to 1550 g, and postnatal age between 10 and 35 days. The newborns were randomized into three groups: patients in group 1 received seven daily doses of 100 U/kg erythropoietin per week; patients in group 2 received two 350 U/kg erythropoietin doses per week; and patients in group 3 did not receive the drug. Hematologic measurements, blood transfusion requirements, and growth rates were followed during therapy.

Results: cases and controls did not differ with respect to weight, length, head circumference, and total time of hospital stay. At the end of the study, no significant difference was observed in the platelet count measurement means, white blood cell count, and ferritin levels in the three groups. However, the final hematocrit and hemoglobin values of patients who did not receive erythropoietin were significantly lower than those of patients who received the drug. The absolute reticulocyte count mean was significantly higher in patients who received erythropoietin after two weeks of treatment when compared with those patients who did not receive the drug. Patients in group 1 and 2 received fewer excessive transfusions (2 or more) than patients in group 3. The administration of 700 U/kg/week erythropoietin significantly reduced the number of excessive blood transfusions. There is no significant difference in blood transfusion volume between patients who received erythropoietin on a daily basis and those who received the drug twice weekly.

Conclusions: the use of erythropoietin did not influence weight gain and growth. The administration of 700 U/kg/week erythropoietin in premature infants with gestational age up to 33 weeks and birthweight up to 1550 g stimulates erythropoiesis and significantly reduces excessive blood transfusion requirements. Erythropoietin showed to be a safe and well tolerated medication, with no short-term side effects in the study population.


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Introduction

Every newborn presents a reduction in red blood cell count during the first weeks of life, which represents a physiological adaptation to the extrauterine environment. In preterm babies, the reduction of hemoglobin (Hb) is faster, and its minimum value is lower when compared to full-term newborns, and this is called anemia of prematurity. The process is self-limited and its intensity increases the more premature newborns are, thus becoming less physiological. The mechanisms involved in anemia of prematurity are: excessive collection of blood samples for lab exams, somatic growth of children, reduced average life of neonatal erythrocytes, small fetoplacental transfusion at birth, and interruption of erythropoietin (EPO) release. Several studies revealed inadequately low serum erythropoietin concentrations during this period. In preterm babies, such suppression persists for weeks, and despite the severity of anemia, erythropoietin is not released before the 30th-34th weeks in most patients.

Blood transfusion is the most commonly used method for the treatment of anemia of prematurity. 80% of approximately 38,000 newborns per year in the United States, with birth weight up to 1,500g, are expected to receive multiple blood transfusions. Even though blood transfusions are carefully carried out, the procedures are not totally safe, and may bring several complications such as hemolysis, hypercalcemia, cardiopulmonary decompensation due to volume overload in extremely small patients, and infections. In addition to these possible complications, early blood transfusions in critically ill newborns cause fetal hemoglobin (HbF) to be replaced with adult hemoglobin type, which shares a weaker affinity with oxygen, promptly carrying it to tissues, thus reducing the stimulus for EPO production.

The effective use of EPO in the treatment of anemia in adults and children with chronic renal insufficiency led to several clinical studies, in which the use of erythropoietin for the treatment of anemia of prematurity was tested. Shannon et al., in 1987, and Rhondeau et al., in 1988, confirmed the presence of erythroid progenitors in the peripheral blood of newborns with anemia of prematurity and normal intrinsic responses to EPO. In 1990, a report of 7 cases showed that EPO associated with adequate doses of iron may accelerate the resolution of anemia of prematurity. In the following year, two clinical randomized studies failed to show improved Hb in patients treated with EPO. Other studies revealed reduced necessity for blood transfusions associated with erythropoietin treatment. Very recently, in 1999, Brown et al. concluded that EPO, when frequently administered, stimulates erythropoiesis in newborns with extremely low birth weight.

This study was carried out in order to assess the efficiency of EPO in the treatment of anemia of prematurity, compare two treatments - daily, and twice-weekly administration of EPO using the same weekly dose - and verify the association between weight and height gain and the use of EPO.

Material and methods

This study was carried out in the Neonatology Unit of the Hospital de Clínicas of Porto Alegre (HCPA). The analysis of the blood samples was performed by the hematology and radioimmunoassay laboratories in this same hospital. The study was approved by the Ethics and Science Committees of the graduate research group of the Hospital de Clínicas of Porto Alegre, and was considered methodologically and ethically adequate according to the Regulating Standards for Research Involving Human Beings. After the assistant doctor’s consent, and the written consent of parents or guardians, the preterm infants who fulfilled the following criteria were included in the study: gestational age up to 33 weeks; birth weight up to 1,550g; postnatal age between 10 and 35 days; platelet count higher than 50,000/mm³; initial Ht above 32%; absence of seizures or brain hemorrhage higher than grade II; no previous history of hemolytic anemia caused by ABO, RH or other incompatibilities, and clinical stability characterized by enteral feeding supplying at least two thirds of daily calorie requirements, absence of congenital or acquired infections, and minimal ventilatory support requirement (defined by up to 30% oxygen, a respiratory rate of 20mvm, 20cm H2O inspiratory pressure, and 4cm H2O expiratory pressure). At this moment, each of the patients was systematically placed into one of the three existing groups: group 1 - patients who received EPO on a daily basis; group 2 - patients who received EPO twice weekly; and group 3 (control group) - patients who did not receive the drug.

Identification data, birth date and weight, and gestational age of each patient were properly registered. The following exams were initially carried out: hemogram, platelet and reticulocyte counts, and ferritin level. The first three exams were repeated every week, and ferritin was dosed every fifteen days. All the blood volume collected for lab exams and all blood transfusion volume and number performed on each patient were also registered. The referral for blood transfusion within the whole study population was made by the assistant doctor of each newborn, and was based on the following criteria: Ht ≤ 20%, inadequate weight gain, three or more apnea or bradycardia episodes within 24 hours, presurgical procedure requirement, disease associated with sudden Ht decline, restoration of the blood collected for lab exams, maintenance of Ht up to 30% associated with minimal ventilatory support requirement, and Ht up to 35% when ventilation requirements are greater. The assistant doctor who recommended blood transfusion did not know to which group the patient belonged. The preterm infants who participated in the study were assessed every day through physical examination, with control of vital signs,
water balance, and weight. Head circumference and length were measured every week.

Recombinant human erythropoietin (rHuEPO) was used in a 2000U/ml concentration. Patients in group 1 received a 100U/kg/day dose of rHuEPO; patients in group 2 received the medication twice weekly in a 350U/kg/day dose; and patients in group 3 did not receive the medication. Medication was administered until the newborn reached 2kg. Therefore, groups 1 and 2 were given the same weekly dose, but in different therapeutic methods. Subcutaneous administration was used. Groups 1 and 2 were given 3mg/kg/day of ferrous sulphate through an orogastric or nasogastric tube from the beginning of the study, and such dose was increased to 6mg/kg/day in the second week of treatment. In group 3, iron supplementation initiated around the 30th day of life according to the assistant doctor’s routine. All the preterm infants received vitamins A, C, D and E, at 2000IU, 35mg, 400IU and 20U daily doses, respectively.

Data collection was interrupted after a sample size calculation revealed that the number of patients studied so far would have to be at least doubled in order to obtain a statistical difference between the two groups of preterm infants who used EPO in different posology. The authors were satisfied when a statistical difference regarding excessive blood transfusions was found between the two treated groups and the control group. An assessment of the study population characteristics was initially made through a descriptive analysis. For the sake of continuous variable analysis, the mean, median, standard deviation, and interquartile range were calculated according to the presence or absence of asymmetric data. For categorical variables, percentage values were obtained. For the comparison of continuous variables between groups, the analysis of variance (ANOVA) was used, with identification of significant differences obtained through Tukey test. The symmetric variables were compared through Kruskal-Wallis test. The categorical and some continuous variables were categorized and compared in contingency tables through the chi-square method. In addition, Pearson correlation coefficients were used to assess the association between categorical variables. Statistical data were analyzed by SPSS (Statistical Package for Social Science) software program. The significance level (alpha = 0.05) was established in all tests.

Results

Forty-five hospitalized newborns were selected between March 1995 and December 1996 from the Neonatology Unit of the Hospital de Clínicas of Porto Alegre after fulfilling all the inclusion criteria. Three of them (one from group 2 and two from group 3) were not included in the data analysis due to intercurrent diseases (2 cases of severe sepsis and one case of necrotizing enterocolitis). Therefore, 42 preterm infants were analyzed: 15 from group 1, 14 from group 2 and 13 from group 3.

The general characteristics obtained through the study are shown in Table 1. All characteristics had a similar distribution between groups, with no statistically significant differences. There was no statistical difference between the number of small and adequate for gestational age newborns. All groups presented clinical stability. The subcutaneous administration of EPO was well tolerated by all patients in the study. No side effects were observed in groups 1 and 2, which received the tested medication.

There was no statistically significant difference between groups with respect to weight gain, length or head circumference. The mean weight at the end of the study was also similar in groups 1, 2 and 3. Table 2 shows the mean weight, length, and head circumference of the three groups, at the beginning and at the end of the study, as well as the average increase of these three variables.

The three groups were similar in terms of calorie and protein supplementation, using an average of 122 cal/kg/day and 2.7g/kg/day, respectively.

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Table 1 - General population characteristics regarding weight, head circumference and length at birth, gestational age, and postnatal age on study admission

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Group 1 n = 15</th>
<th>Group 2 n = 14</th>
<th>Group 3 n = 13</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Weight at birth (g)</td>
<td>1191.1</td>
<td>261.45</td>
<td>1173.93</td>
<td>272.24</td>
</tr>
<tr>
<td>Gestational age (weeks)</td>
<td>31.33</td>
<td>1.88</td>
<td>31.71</td>
<td>1.60</td>
</tr>
<tr>
<td>Initial weight (g)</td>
<td>1181.3</td>
<td>219.3</td>
<td>1164.3</td>
<td>268.8</td>
</tr>
<tr>
<td>Initial length (cm)</td>
<td>37.9</td>
<td>2.86</td>
<td>37.44</td>
<td>2.78</td>
</tr>
<tr>
<td>Initial head circumference (cm)</td>
<td>26.93</td>
<td>1.66</td>
<td>27.36</td>
<td>2.05</td>
</tr>
<tr>
<td>Initial age (days)</td>
<td>16.13</td>
<td>6.97</td>
<td>14.42</td>
<td>5.54</td>
</tr>
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</table>
Table 3 shows the mean values for Ht, Hb, reticulocyte, total white blood cell count, platelet and serum ferritin of the three groups at the beginning of the study. All these variables were statistically similar at the time mentioned.

Table 4 shows the mean values for Ht, Hb, ferritin and reticulocytes of the three groups at the end of the study, and for reticulocytes at the end of the second week of treatment.

The result of the final Ht mean value analysis revealed a significant difference between patients who received EPO and those who did not receive the medication. In group 1 and 2, patients who received erythropoietin daily or twice weekly, respectively, the final hematocrit mean value was significantly higher if compared to that obtained in group 3 (control group).

Regarding Hb mean value at the end of the study, the smallest value was found in group 3: 8.3. The statistical analysis showed a significant difference between the groups. A significantly higher reduction in Hb is observed in patients who did not receive the tested medication (group 3) in comparison with groups 1 and 2.

There was no significant difference as to the total white blood cell count between the groups in any of the analyzed weeks. There was no difference as to neutrophil count between the treated groups (1 and 2) and the group that did not receive the medication (group 3). Neither was there any significant difference as to platelet count between the groups in any of the analyzed weeks.

Statistical tests were carried out to assess ferritin levels. The first screening did not show any significant difference between the groups in any of the analyzed weeks. As the variable is asymmetric, nonparametric tests were performed in order to confirm the obtained result. Nevertheless, the result obtained with the first screening - the absence of significant difference - was still 0.344 (nonparametric test P value).

The absolute reticulocyte count mean value at the end of the second week of treatment was significantly higher in patients who received the medication when compared to those who did not receive the drug. There was no statistically significant difference as to the mean values for this variable at the end of the study.

As far as blood samples are concerned, an average of 21.9 ml, 22.9 ml and 25.1 ml of blood were collected in groups 1, 2 and 3, respectively, throughout the study. There was no statistically significant difference among the three groups (P=0.839).

The blood transfusion volume was 4.6 ml per patient in group 1; 9.6 ml in group 2, and 17.6 ml in group 3. Although group 3 (control group) tended to receive a higher blood transfusion volume per patient, these findings were not statistically significant (P=0.156).

The mean value for the number of blood transfusions per patient was 0.33 in group 1, 0.64 in group 2, and 1.62 in group 3 (P=0.091). There seems to be a difference between the groups; however, the established significance level (alpha 5%) was not attained.

The excessive blood transfusion variable, defined as two or more blood transfusions per patient, revealed a significant difference between the groups (P=0.043). Excessive blood transfusion was considered as more than one transfusion per patient based on the exposure to multiple donors (more than one donor per newborn). In group 1, only one (6.7%) out of the 15 patients who received the medication on a daily basis needed excessive blood transfusion. In group 2, three (21.4%) out of the 14 patients who used the medication twice weekly were submitted to excessive blood transfusion. In group 3, which did not receive erythropoietin, 5 (38.5%) out of the 13 newborns required excessive blood transfusion. Although children in group 1 had a smaller number of excessive blood transfusions

<table>
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</tr>
</thead>
<tbody>
<tr>
<td>Final weight (g)</td>
<td>2,012.7 ± 45</td>
<td>1,980.7 ± 73.8</td>
<td>2,005.4 ± 43.5</td>
<td>0.290</td>
</tr>
<tr>
<td>Weight gain (g/day)</td>
<td>24.7</td>
<td>24.3 ± 4.2</td>
<td>26.2 ± 6</td>
<td>0.652</td>
</tr>
<tr>
<td>Final length (cm)</td>
<td>42.8 ± 1.3</td>
<td>42.5 ± 1.2</td>
<td>42.2 ± 1.01</td>
<td>0.409</td>
</tr>
<tr>
<td>Increase in length (cm)</td>
<td>6.3 ± 3.8</td>
<td>5 ± 2.4</td>
<td>4.6 ± 3</td>
<td>0.347</td>
</tr>
<tr>
<td>Final head circumference (cm)</td>
<td>31.5 ± 0.9</td>
<td>31.8 ± 1.08</td>
<td>31.6 ± 0.64</td>
<td>0.585</td>
</tr>
<tr>
<td>Increase in head circumference (cm)</td>
<td>4.6 ± 1.8</td>
<td>4.5 ± 2.2</td>
<td>4.6 ± 2.2</td>
<td>0.981</td>
</tr>
</tbody>
</table>
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Discussion

A new concept of and approach to the treatment of anemias, including anemia of prematurity, was achieved through a more detailed knowledge about hematopoiesis in fetal and neonatal life and about its regulating factors, especially EPO. EPO, a cytokine whose function is to stimulate erythropoiesis, has been available for use and clinical studies since 1985; the use of EPO was expected to eliminate the necessity for blood transfusions.19

In our study, we had an initial concern with the assessment of the characteristics inherent to the study population. The fact that these characteristics had a similar distribution among the three groups, as shown in Table 1, minimizes occasional selection biases that might interfere with the obtained results.

The three groups were similar in terms of weight and gestational age adjustment. Most preterm infants who participated in our study were appropriate for gestational age. This information is extremely important as, according to Brown et al.,16 gestational age, especially under 30 weeks, is the best prediction value for blood transfusion necessity after the second week of life. Therefore, the likelihood that a small for gestational age newborn will need blood transfusion is theoretically remote.

All patients presented clinical stability, whose criteria were described in Material and Methods, and in most publications.1-3,9,22,27-29 Although some studies did not

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</thead>
<tbody>
<tr>
<td>Hematocrit (%)</td>
<td>Mean</td>
<td>Mean</td>
<td>Mean</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>SD</td>
<td>SD</td>
<td></td>
</tr>
<tr>
<td>Hemoglobin(g/dl)</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Total leukocytes</td>
<td>12.120</td>
<td>10.550</td>
<td>12.720</td>
<td></td>
</tr>
<tr>
<td>Reticulocytes (%)</td>
<td>2.45</td>
<td>2.12</td>
<td>3.02</td>
<td></td>
</tr>
<tr>
<td>Platelets</td>
<td>381.266</td>
<td>392.643</td>
<td>490.770</td>
<td></td>
</tr>
<tr>
<td>Ferritin (ng/ml)</td>
<td>422.5</td>
<td>269</td>
<td>501.5</td>
<td></td>
</tr>
</tbody>
</table>

Table 4 - Means and standard deviations regarding hemocytometric values at the end of the study: Hematocrit, Hemoglobin, Ferritin, and Reticulocytes in the second week of treatment

<table>
<thead>
<tr>
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<tbody>
<tr>
<td>Hematocrit (%)</td>
<td>Mean</td>
<td>Mean</td>
<td>Mean</td>
<td></td>
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<tr>
<td></td>
<td>SD</td>
<td>SD</td>
<td>SD</td>
<td></td>
</tr>
<tr>
<td>Hemoglobin (g/dl)</td>
<td>9.5</td>
<td>9.3</td>
<td>8.3</td>
<td></td>
</tr>
<tr>
<td>Ferritin (ng/ml)</td>
<td>146.5</td>
<td>93.5</td>
<td>210.2</td>
<td></td>
</tr>
<tr>
<td>Reticulocytes at the end of the study (%)</td>
<td>10</td>
<td>9.3</td>
<td>6.8</td>
<td></td>
</tr>
<tr>
<td>Reticulocytes in the second week of treatment (%)</td>
<td>11</td>
<td>10.9</td>
<td>4.1</td>
<td></td>
</tr>
</tbody>
</table>
establish a correlation between Ht and respiratory manifestation\(^4\), the maintenance of Ht at 40% in patients with respiratory diseases was one of the criteria established by Strauss et al.\(^{30}\) in 1990, to indicate blood transfusion necessity. Therefore, if there were theoretically an increased clinical and ventilatory discrepancy between the groups, e.g. presence of newborns with a greater necessity for ventilatory support in groups 1 and 2, which received EPO, one would wrongly conclude that the tested medication does not reduce the number of blood transfusions since patients in these groups would be submitted to blood transfusions more frequently due to their critical health status.

The weight and height gain of newborns throughout the study was similar for all three groups, matching the results described in the literature.\(^1,3,24\) There were no statistically significant differences in terms of weight, length, and head circumference mean values at the end of the study, similarly to what Bechensteen et al. described in 1993\(^{1}\) and in 1996.\(^{31}\) There were no differences, at any moment, with respect to weight gain, length and average head circumference between the groups that received EPO and the group that did not receive the drug. The reason for such observation is not clear. It is known that by stimulating an increase in erythropoiesis, there will be an increase in vitamin and protein requirements, as these are not properly provided by the diet received by newborns. Although Shannon et al.\(^{29}\) showed, in 1990, a faster weight gain in patients treated with EPO, most clinical studies\(^1,3,10,24,27\) did not present a significant difference in terms of weight gain between the treated groups and the control group. The daily weight gain in groups 1, 2 and 3 in our study was respectively 24.7g, 24.3g and 26.2g, similar to what Meyer et al. obtained in their study: weight gain between 23.2g (patients treated with EPO) and 22.7g (newborns who did not receive the medication). Bechensteen et al. obtained an average gain of 18.5g/day. In our study, all preterm infants were formula-fed (adequate diet regimen for preterm infants), and received an average of 121.8 cal/kg/day. Halperin et al.\(^{7}\) did not find statistically significant differences as to the weight gain variable.

By analyzing the initial hematological values - Ht, Hb, reticulocyte, total white blood cell and platelet counts, and ferritin levels in newborns, as shown in Table 3, no statistically significant differences were found between the groups. Therefore, the differences observed in the final Ht and Hb counts can not be explained through the initial counts of these variables. The results reveal that final Ht counts were significantly higher in patients who received EPO, either daily or twice weekly, in comparison with the control group, which is in agreement with several studies in the literature.\(^1,3,17,24,28\)

The results obtained clearly show that the absolute reticulocyte count is significantly higher at the end of the second week in patients who received the medication in comparison with those who did not receive the drug, which is an evidence that preterm infants are able to respond to exogenous EPO by increasing erythropoiesis. Bechensteen et al.\(^1\) found similar results. The peak reticulocyte count was obtained at the end of the second week of treatment, with a statistically significant difference between initial reticulocyte count and the values obtained at the end of the second week of treatment. Such findings were reported by the authors of the present study and by other researchers as well.\(^16,22\) When we assessed this variable at the end of the study, we did not find any statistically significant difference between the groups although reticulocyte count tended to be lower in group 3. One of the possible reasons for that is an interrupted production of endogenous EPO, resulting from an increase in Hb or, more precisely, the absence of Hb reduction that is necessary for EPO stimulation.

There was no significant difference between the groups in terms of total white blood cell and platelet counts, in any of the analyzed weeks, as already mentioned by some authors.\(^1,3,9-10,27,32\)

In Brazil, as in the United States, there is no agreement as to the use of EPO in the treatment of anemia of prematurity. According to Doyle et al.,\(^{33}\) clinical studies on EPO were aimed at assessing preterm infants, usually with gestational age up to 34 weeks, and birth weight up to 1,500g; however, ages varied at the beginning of the treatment. In some studies,\(^1,3,18,24,28-29\) EPO administration occurred at an early date, starting in the first week of life while in other studies,\(^1,3,18,24,28-29\) administration took place at a later date, on average 21 days of life. In this study, the average age of treatment initiation was approximately 15 days. The beginning of treatment around the 2\(^{nd}\) and 3\(^{rd}\) weeks of life and its continuation during 6 to 8 weeks seems to be the most effective method according to the literature. On the other hand, the usefulness of EPO administration within the first hours of life in order to reduce blood transfusion requirements in the first 15 days of life has not been clearly established yet.

The ideal therapy approach for EPO administration remains undetermined due to different weekly doses and their frequency in these studies.\(^6\) Doses range between 150-1400U/kg/week, and EPO usage frequency varies from 2 to 7 days a week. The authors agree that EPO administration at doses equal or higher than 500U/kg/week does not seem to cause any complications, induces marked stimulation of erythropoiesis, and significantly reduces blood transfusion requirements in newborns. Halperin et al.\(^9\) in 1992, found dependency of dose response; there is, however, uncertainty about the existence of a positive effect on erythropoiesis through a more frequent administration of EPO. Although Brown et al.\(^{26}\) have shown such effect very recently, there is neither a significant reduction in the number of blood transfusions in newborns nor a difference between Hts of the group that received EPO at shorter intervals (5 times a week) in comparison with the group that received EPO just
twice weekly. A statistical analysis was carried out in groups 1 and 2 in order to compare daily and twice-weekly EPO administrations. No significant difference was obtained between the groups, probably due to small sample size, although preterm infants in group 1 showed a lower blood transfusion frequency (6.7%), two or more transfusions per patient than newborns in group 2 (21.4%). In order to obtain a significant difference between both groups of preterm newborns regarding the number of excessive blood transfusions, it is necessary to have, at least, a sample size almost twice the one assessed in our study- 25 patients per group.

The efficient use of EPO in reducing the number of blood transfusions in preterm infants was shown in several clinical studies.1-3,10,17,22,23,26-29,32 In this study, statistical analysis did not present a significant difference between the groups, although the data indicate that patients who were not treated with EPO tend to require a higher blood transfusion volume. On the other hand, fewer patients treated with EPO twice-weekly were submitted to excessive blood transfusions in group 2, compared to patients in group 3, who did not receive the medication (21.4% versus 38.5% respectively) - a statistically significant difference. EPO is very useful but should not be used isolatedly when trying to reduce the exposure of newborns to blood transfusions. There is an urgent necessity to establish transfusion criteria, through which blood transfusions are only performed in extremely necessary cases, and when their benefits are clearly evident. The collection of blood samples for lab exams should also be restricted. Exams should be rationally required and, whenever possible, microtechniques for blood sample collection should be employed.

Erythropoiesis, at any age, depends on adequate nutrition, especially on protein and iron intake; protein and iron deficiencies are considerable limiting factors for erythropoiesis.1,34 As patients at this stage present a faster growth than at any other stage of their lives, adequate nutrition is of paramount importance.33 Taking this fact into consideration, we were concerned with offering newborns a proper milk supplementation. As previously mentioned, the analysis of calorie and protein supplementation did not reveal any significant difference among the three groups. Perhaps, a higher protein intake, 3g/kg/day, should be recommended as pointed out by Bechenteen et al.1 It is advisable to adjust protein intake according to the amounts recommended for newborns expected growth, even though the adequate amount of protein for the stimulation of erythropoiesis during EPO treatment has not been defined.34 With respect to iron intake, the same authors used 18mg/day of iron, increasing it to 36mg/day in the event of a reduction in iron concentration.34 The authors state that erythropoiesis is enhanced due to high protein intake, since a relatively low dose of EPO (300U/kg/week) was used. In our study, we did not use as high iron and protein supplementation as that used by Bechenteen et al.35 We used a 700U/kg/week dose of EPO, more than twice the dose administered by Bechenteen et al.,35 and the increase in reticulocyte count at the end of the second week of treatment as well as Ht and Hb values at the end of the study were higher for the groups treated with EPO if compared to control groups.

Preterm infants have low body iron stores due to the reduction in gestational period and low iron intake during the long-term hospital stay to which they are submitted. The use of EPO was shown to increase iron deficiency in such a way that iron supplementation becomes a necessity during EPO treatment. The nonuse of iron was related to therapeutic flaws, and low supplementation doses led to body iron store depletion to refractory anemia.27 Currently, we can affirm that the amount of iron supplementation that is necessary for an effective high-dose EPO treatment should not be lower than 6mg/kg/day.18,27 Clinical studies present varied iron supplementation doses, thus interfering with this interpretation. Suggestively, the available amount of iron might be a determining factor for EPO administration.18 The aspects related to routes of administration and appropriate moment for introducing iron supplementation are still under consideration. Most articles previously mentioned in our study use oral administration of iron at 2 to 8mg/kg/day. Ohls et al.32 used the intravenous route for iron supplementation combined with total parenteral nutrition. A study carried out by Meyer et al.36 revealed that the oral or parenteral administration of iron at high doses was sufficient to maintain erythropoiesis at stable levels despite a more depleted body iron store in the group that received iron orally. Based on this information, the authors of this study suggest intravenous administration of iron for newborns who have extremely low ferritin levels.

After assessing the results obtained from the studies carried out by Brown et al.,26,34 we regarded ferritin levels as more reliable than serum iron or transferrin saturation levels. The highest ferritin levels obtained through our study were observed in the control group (with no significant difference), and probably resulted from the mobilization of iron by newborns in the group treated with EPO. As previously shown by results, there was an expected decline of this variable throughout the study, but no statistical difference was observed among the groups, which is in agreement to the findings of some authors.23,27,34,35 This finding may be explained through adequate iron supplementation, which prevented iron contents from considerably declining during EPO treatment.

In our study, and in most clinical studies with newborns affected by anemia of prematurity,1,9,16,17,22-24,27-29,31,34,35 the standard route for the administration of EPO was the subcutaneous route.32 The intravenous route was only used by a few authors.32,37

The possible side effects of EPO such as thrombocytopeny and granulocytopenia should not be overlooked. No toxic effect caused by EPO administration was shown in our study, which is in agreement with the
findings of other authors. Conclusive data on long-term effects of EPO administration were not obtained, and there is no prospective study up to now concerned with these effects.

We conclude that the administration of EPO at 700U/kg/week in preterm infants with birth weight up to 1,550g and gestational age of 33 weeks stimulates erythropoiesis and considerably reduces the number of excessive blood transfusions, minimizing the exposure of newborns to their potential risks. Based on the results obtained through our study and on reviewed literature, we recommend the routine use of EPO in preterm infants with gestational age up to 33 weeks, and birth weight up to 1,550g, who have clinical stability. The subcutaneous administration of EPO should be initiated around the 15th day of life at 700U/kg/week. A more comprehensive study population needs to be assessed so that we can set up an ideal EPO dosage. The use of EPO, at the recommended dose, was safe and well tolerated.

References


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