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Treatment with erythropoietin in neonatology

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ABSTRACT

The article presents the basics and control of erythropoiesis in the fetus and the newborn, the development of anaemia of prematurity and its treatment, with an emphasis on the use of human recombinant erythropoietin. The Intensive Care Unit of the Paediatric Clinic Maribor began treating anaemia of prematurity with erythropoietin in 2000. After introducing the treatment, the clinic found that the number of blood product transfusions and the needed blood volume decreased. In addition to erythropoietin, this was the result of stricter criteria for applying transfusion of concentrated erythrocytes.
Key words: preterm infant, anaemia of prematurity, erythropoietin, transfusion

INTRODUCTION

After birth, the transition from a hypoxic to a hyperoxic state in all newborns lowers haemoglobin (Hb) concentrations. Improved oxygenation lowers erythropoietin (EPO) concentrations, which in the neonate newborn leads to physiological, mostly asymptomatic anaemia between 8 and 12 weeks of age. (1-4) Anaemia of prematurity is the more expressed, deficient response of a premature newborn to this transition. It manifests itself as a normochromic anaemia, which in addition to a severely reduced concentration of Hb is characterized by low EPO serum levels.
Many preterm newborns experience anaemia in the first few days of life. Reasons range from a low starting level of Hb (compression, the separation of the umbilical cord stump or bleeding), or multiple venepunctures for laboratory tests and erythrocyte haemolysis because of infection. In all three cases, the condition is referred to as a pathological (non-physiological) anaemia. Physiological anaemia of prematurity is a condition characterized by the temporary absence of an appropriate erythropoietic response to hypoxia. The rapid growth and increased blood volume in preterm infants are not met by a corresponding increase in erythrocyte mass. (5-10)
In preterm infants, this physiological drop in Hb happens after the first week. The most significant factor behind non-physiological anaemia is insufficient excretion of EPO from the immature cells of the renal interstitium - the transition from the liver to renal production of EPO is late because it is not determined by chronological age but by gestational age (time from fertilization). (11-14) In the first few months, preterm infants thus depend on the formation of EPO in the liver; however, because of their overly low sensitivity to hypoxia, the production of EPO is insufficient in spite of low Hb values. Particularly in children with a chronic lung disease, erythropoiesis is inhibited by many inflammatory mediators (IL1, TNF) and medications disrupting the function of EPO (corticosteroids).
The non-physiological anaemia of the preterm infant, better known as the anaemia of prematurity (4-12 weeks after birth) appears mostly in preterm infants with very low birth weight (1,500 g or less) and is a time-limited, hyporegenerative, normocytic normochromic anaemia with decreased erythropoiesis in the bone marrow reflected in very low reticulocyte values in peripheral blood (under 3.0 %), while leukocyte and thrombocyte values are generally normal. (15-17)
Haemoglobin concentration is under 70-100 g/L, haematocrit is under 0.30; the child also has clinical signs of anaemia such as poor weight gain, tiredness at feeding, tachypneas, dyspneas, tachycardia, apnoea attacks and metabolic acidosis.
Hypoperfusion of the intestines can cause necrotizing enterocolitis, BDP treatment is slowed down, as well as closing of the ductus Botalli.
The most worrying effect is the possibility of permanent hypoxic damage (particularly of the central nervous system).

ERYTHROPOIETIN

Erythropoietin (EPO) is the product of the gene in the middle of the long arm of chromosome 7 (g22) and is the only hematopoietic growth element functioning as a hormone.
In terms of its chemical composition, EPO is a sialoglycoprotein, with a molecular mass of 35-39 kD.
The main part of the molecule is the polypeptide chain (166 amino acids) which is key in the interaction between EPO and the receptors of the target cell.
In the fetus, EPO is mostly created in the liver, which is in a relatively hypoxic condition for most of the duration of a pregnancy because most blood rich in oxygen bypasses the liver and flows from the placenta through the umbilical vein and the ductus venosus directly into the heart. However, because of the low sensitivity of the liver "oxygen sensor's" to hypoxia, EPO creation is low and does not cause polycythaemia. Only in the last few weeks of pregnancy is the hormone created in the kidney, where the oxygen sensor is considerably more sensitive to hypoxia (18-24).
After birth, most EPO is synthesized in the peritubular interstitium cells of the renal cortex; less than a fifth is created in the liver perivenular hepatocytes and Kupffer cells and in the macrophages. EPO that is excreted as a reaction to hypoxia travels with blood to the target hematopoietic organ, where it connects to specific receptors at the pre-stages of erythrocytes and thus causes them to divide and mature.
Using recombinant DNA technology, hu-

man recombinant EPO was first synthesized in 1985. It was first successfully used in treating adults with anaemia caused by chronic renal failure. The first pilot study is the use of human recombinant EPO for anaemia of prematurity was published by Halperin et al. in 1990. In the pilot studies, doses for adults were initially used. These doses were too small for preterm infants because of their higher distribution volume and faster excretion of the medication, which reduced the efficiency of EPO. Later studies, in which higher doses of EPO were used, proved that EPO successfully triggers erythropoiesis. The best administration method for preterm infants has not yet been determined; both subcutaneous and intravenous application are possible. EPO is sometimes administered by continuous infusion or as a supplement in parenteral nutrition. It is still unclear when to begin treatment with EPO. Cochrane's database from 2006 provides a review of articles about early and late treatment with EPO. 2,000 preterm infants from 23 studies were included in an analysis of early treatment with EPO (before 8 days of life). An increased risk of grade 3-4 retinopathy was found, which is why routine treatment with EPO is not recommended. 1,300 preterm infants from 28 studies were included in an analysis of late treatment with EPO (between 8 and 28 days of life). (25-30) The number of transfusions decreased and the volume of administered blood decreased significantly. In spite of statistically significant differences the clinical value is not that great because most preterm infants are administered transfusion prior to treatment with EPO. EPO doses of between 50 and 1,200 IE/kg of body weight per week were used in the published studies. They were divided into 3-5 doses, and treatment lasted 3-8 weeks. (31-36)

In none of the studies did treatment with rHuEPO suppress the excretion of the body's own hormones and also did it influence the transformation of fetal Hb into the adult form. To date, no study of preterm infants has shown sensibilisation or the development of antibodies to rHuEPO formulae. Because of stimulating erythropoiesis, treatment with EPO must be supplemented with iron; if not, higher production of Hb causes iron deficiency or lowers ferritin in the serum. Many studies list various recommended doses of iron, ranging from 1 mg/kg/day to 10 mg/kg/day. (37-40)

Views and recommendations on the use of EPO still vary, even though it has been used for preterm infant anaemia for over 10 years. An overview of studies in Cochrane's database from 2012 does not show a significant reduction in the need for transfusion, nor improvement in the clinical outcome in case of late administration of EPO (41). In contrast, a recent randomized control study with 102 extremely premature neonates proved once again that the use of EPO significantly reduces the number of transfusions and thus the number of various donors for each child since more than half of the patients required no transfusion at all! (42,43)

Also important is the neuroprotective effect of EPO on the preterm infants' brain; however, this applies in significantly higher doses, which was examined in studies on animals and humans.

Our experience

The Intensive Care Unit at the Paediatric Clinic of the University Clinical Centre Maribor introduced EPO treatment in 2000. The unit follows the Slovene national guidelines which are as follows:

- Preterm infants with a birth weight below 1,500 g are treated with a dose of 250 IE/kg 3 x week;
- Treatment begins at 3 to 5 days of age and is completed within the corrected gestation al age of 37 weeks;
- EPO treatment is supplemented with iron. The dose depends on the preterm infant's age and transferrin saturation.

To get a better view of the effectiveness of treatment with EPO, the documentation of patients treated at our unit was examined for preterm infants born before the 32nd week in two different time periods between 1997-1999. For the period in which treatment with EPO was not routine, the documentation of 28 preterm infants was analysed. For the second period between 2004-2006, in during which treatment with EPO was well established, documentation of 60 preterm infants was analysed. We wanted to establish the effect of treatment with EPO on the number of transfusions, the volume of blood administered and the haematocrit value at discharge.

As expected, it was found that, owing to an increase in the number of surviving extremely premature infants, the average gestation age and average birth weight in the first group were slightly higher.

Only individual preterm infants (28.6 %) were treated with EPO in the first period; in the second, as many as 81.7 % of preterm infants born before the 32nd week of gestation were treated. The main objective was to find whether the number of transfusions and the volume of blood administered decreased after introducing treatment with EPO. The results show that the number of transfusions per individual preterm infant decreased significantly (from 4.5 to 2.2) after treatment with EPO was introduced. In addition, the volume of blood administered decreased (from 58.0 to 32.2 ml/kg).

BLOOD TRANSFUSIONS IN THE TREATMENT OF ANAEMIA IN NEWBORNS

No uniform instructions exist for treating anaemia of prematurity with blood transfusion. Most protocols regulating the decision when to administer transfusion of concentrated EPO considers factors such as the need for mechanical ventilation, presence of serious illnesses (sepsis, BPD). One of the more frequently listed protocols in the treatment of anaemia is the Shannon protocol from 1995.

Table 1. Overview of the average number of transfusions, volume of administered blood and Ht and reticulocyte values at discharge for the period 1997-99 and 2004-06.

	Number of transfusions	Volume of blood ml/kg TT Haematocrit		Reticulocytes 109/l
1997-1999	4.5	58.04	0.34	/
2004-2006	2.25	31.22	0.34	108.9

Table 2. Indications for administering transfusion of concentrated EPO in treatment for anaemia of prematurity (after Shannon et al. 1995)

HEMATOCRIT <0.35
- % oxygen in the inhaled air in the incubator > 35 %
-child on CPAP or artificial ventilation with mean pressure > 6 cm H2O
HEMATOCRIT < 0.30
- % oxygen in the inhaled air in the incubator > 35 %
-child on CPAP or artificial ventilation with mean pressure < 6 cm H2O
-clinically significant apnoea or bradycardia attacks (> 9 episodes in 12 hours or 2 episodes in 24 hours that require ventilation with a mask) when administering therapeutic doses of methylxanthine preparations
-tachycardia (>180 / minute) or tachypnea (>80 / minute) that lasts 24 hours
-increased body weight < 10 g/day in the period of 4 days at energy intake > 100 kcal/kg/day
-if the child needs a surgical intervention
HEMATOCRIT < 0.20
-if no clinical signs of anaemia exist and the number of reticulocytes < 100x109/l
TRANSFUSION IS NOT ADMINISTERED
-only to replace blood drawn for laboratory tests, and because of low Htc values

Treatment with transfusion does not always improve a child's condition and can always involve complications (chance of infection, reaction to transfusion, disruption in the functioning of the immune system, fluid or iron overload).

Table 3. The number and proportion of transfusions in preterm infants born before the 32nd week of gestation (WG) for the periods 1997-1999 and 2004-2015 in three-year cohorts

Year	Number of newborns	Transfusion in all neonates; n (%)	Newborn < 32 WG; n (%)	Proportion of transfusions (%) in newborns <32 WG
1997-1999	5769	93 (1.6%)	79 (1.4%)	73/88 (83%)
2004-2006	6082	84 (1.4%)	118 (1.9%)	79/118 (67%)*
2007-2009	6453	105 (1.6%)	111 (1.7%)	87/111 (78%)*
2010-2012	6787	105 (1.5%)	133 (1.9%)	96/133 (72%)
2013-2015	6536	102 (1.5%)	133 (2.0%)	93/133 (70%)

* $p < 0,05$ – change in comparison to the previous period

In the period under observation, the number of preterm infants <28 WG began to increase after 2006, when their number doubled. After a significant drop in the number and proportion of transfusions following the introduction of therapy with EPO in the period 2004-2006 in the group of preterm infants <32WG, their number again increased in the period 2007-2009, which is mostly connected with a higher proportion of very premature newborns.

CONCLUSION

The use of rHuEPO in neonatology has so far proven that the medication enhances erythropoiesis, as indicated by the reticulocytosis. It prevents the decrease and then increase of the haematocrit or the concentration of haemoglobin. Although existing studies mostly show the advantages of rHuEPO (its neuroprotective effect has also been proven), its long-term effects remain unknown.

The use of rHuEPO for preventing anaemia is advisable for preterm infants with a very low birth weight (<1,000 g) and a gestation age of under 32 weeks. The recommend is subcutaneous administration 3-5 times a week of 250 IE/kg (epoetin alfa-Eprex ali epoetin beta-Neorecormon) or 100 IE/kg (epoetin omega-Epomax). Duration of administration depends on the gestation age and clinical condition. Mandatory is parallel administration of iron of 3-5 mg/kg/day with a supplement of Vitamin E. In case of a weak reaction it is necessary to verify the level of ferritin in the serum and increase the intake of iron if needed.

Our results indicate that the number of transfusions and the volume of blood administered at our department decreased after introducing treatment with EPO, even though the unit is yet to achieve the desired results of Ohl's recent study, where more than half of the most premature infants did not require transfusion at all. The good results at our clinic were made possible not only because of treatment with EPO; also important were stricter criteria for treatment with blood and the effort by all Intensive Care Unit staff to reduce blood collection to a minimum during everyday work.

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