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## Preoperative Use of Erythropoietin as a Means of Blood Saving in Children with Nonsyndromic Craniosynostosis

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*It is known that neurosurgical operations are characterized by blood loss requiring hemotransfusion. At the same time, world practice oversees a tendency towards restricted use of donor blood components for this purpose. This has become possible through the use of various blood saving methods, including use of erythropoietin-based drugs. This review is dedicated to analysis of the modern research data on preoperative use of erythropoietin-based drugs in combination with iron preparations in patients with nonsyndromic craniosynostosis requiring surgical correction. It has been demonstrated that preoperative (2-3-week-long) administration of erythropoietin allows limiting hemotransfusion and reducing the amount of intraoperatively transfused donor blood. Based on these data, we assume that erythropoietin is an effective and safe alternative to the “standard” means of blood saving, such as normovolemic hemodilution, controlled hypotension, automatic blood sampling and reinfusion of the patient’s erythrocytes.*

**Keywords:** children, craniosynostosis, hemotransfusion, erythropoietin, blood saving.

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### Relevance

Craniosynostosis is a disease characterized by premature fusion of one or more cranial sutures and restriction of skull volume with the skull acquiring abnormal shape. Because of insufficient volume of skull cavity in the active growth period, a non-specific brain injury occurs, intracranial hypertension and secondary neurological disorders develop. Syndromic and nonsyndromic (NsCS) forms of craniosynostosis are distinguished. The latter are responsible for 80-85% of all the cases [1]. Population frequency of NsCS, according to different data, ranges from 0.3 to 1.4 cases for 1000 newborns [2].

This pathology can only be treated surgically, therefore it is important to use the less traumatic surgical methods [3]. One of such methods is endoscopic craniectomy. The peculiarities of surgical treatment of different forms of NsCS and anesthetic support have already been described in the Russian research literature [4, 5].

Previously it was considered that in any surgical operations intraoperative blood loss is recovered both with respect to the blood volume and its quality characteristics. For this purpose, donor blood preparations are used, with the help of which the transport function of blood is restored with maintenance of an adequate level of hemoglobin [6, 7]. Previously it was assumed that the larger the bleeding volume is, the more intensive hemotransfusion should be carried out [8, 9]. However, given the constant threat of increase in post-transfusion complications (both infectious and non-infectious), high cost of hemotransfusion media, fast reduction of the number

of donors, the opinion on transfusion of donor blood has radically changed [10]. One is to remember that post-transfusion complications in children develop faster and more often than in adults [7].

According to some data, up to 40-66% of allohemotransfusions are not justified. It is due to the fact that during the long conservation of donor blood preparations, erythrocytes lose their elasticity and osmotic resistance are prematurely destroyed in the microcirculation channel (sludge syndrome), aggravating the increase of ischemia. Because of that, the risk of an onset of ischemia of organs and tissues associated with use of hemotransfusion in most cases exceeds the possible positive effect [11].

### **The existing methods of blood saving as an alternative of hemotransfusion**

Today, a trend towards the restriction of the use of hemotransfusion is observed in world practice, and therefore transfusion of any donor components requires serious indications [7, 12, 13].

In Russia and abroad, methods of blood saving, alternative to donor blood transfusion are used: normovolemic hemodilution, manageable hypotension, self-administered blood collection and autohemotransfusion, reinfusion of the patient's own washed erythrocytes – [14] ones.

Use of drug manageable hypotension as a method of blood saving during surgery in children does not prevent an increase in complications occurring as a consequence of reduction of perfusion pressure in brain, heart, and kidney vessels.

Self-administered blood collection is accompanied by sharp fluctuations of circulating blood volume with the development of the body's metabolic response to stress (activation of the adrenal system, increase of vasoconstriction, direct loss of protein, increase of catabolism, increase of oxygen intake). In addition, blood collection is accompanied by pain and negative emotional impact [15].

In pediatrics, 2 basic methods are widely applied: acute normovolemic hemodilution and reinfusion of the patient's own washed erythrocytes. The use of hemodilution when the calculated amount of donor blood is substituted with hydroxyethyl starch drugs (refortan, voluven), leads to increase in acute or subacute anemia, reduction of blood viscosity, while preserving tissue perfusion. Use in infants (up to 1 year) is not recommended, but nevertheless the procedure is carried out and yields the positive result of a decrease in hemotransfusion volume, especially in reconstructive operations for craniosynostosis correction [16]. The negative side of this is the development of hypocoagulation that requires dynamic control for prevention and correction of coagulation hemostasis disturbances [17].

In pediatric neurosurgery with massive blood loss prognosis (over 50% of the circulating blood volume) during the operation and for decreasing the number of hemotransfusion in the early postoperative period reinfusion of the patient's own erythrocytes washed with C.A.T.S system is used as a method of blood saving. With the application of this method, loss of blood coagulation factors occurs and coagulation system disturbances may develop [17, 18].

Some authors believe that the volume of acceptable blood loss can be increased with preoperative increase in erythrocyte, hemoglobin, and hematocrit contents with by means of drug-induced stimulation of erythropoiesis with erythropoietin drugs, thus contributing to the reduction of frequency of the use of hemotransfusion therapy [19, 20].

### **Erythropoietin and erythropoiesis stimulation**

In 1906, the French scientists R. Carnot and S. De Flandre first expressed an assumption about the possible existence of a hormonal factor controlling erythropoiesis in the human body. They called it hemopoietin (erythropoietin) [21]. The hormone was isolated in substance in 1977. [22] In 1985, F.K. Lin and K. Jacobs et al. cloned the erythropoietin gene [23], and the first clinical

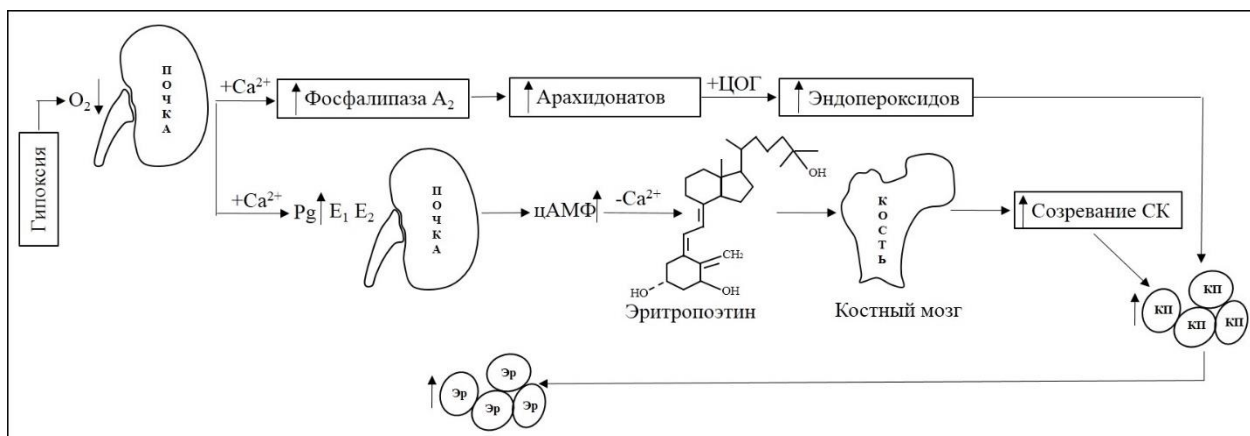
trials of recombinant erythropoietin were conducted in 1986–1987. The possibility to effectively and safely regulate erythropoiesis appeared [24, 25].

Erythropoietin (in Greek, *erythrós* means 'red', *poietikós* means 'forming') is a glycoprotein composed of a chain of 165 amino acids and the carbohydrate part [26, 27]. The latter is formed by a chain of O-linked and three chains of N-linked oligosaccharide chains at the ends of which the sialic groups are situated providing for the biological activity of the whole molecule. Erythropoietin has the molecular mass of 30.4 kDa. The biological semi-decay of the molecule is 6-8 hours. The blood level of erythropoietin in healthy people varies within the range of 0.01-0.03 I.U./ml [28].

Erythropoietin is the basic hematopoietic hormone synthesized by the kidneys (up to 90 %) and liver. It regulates various stages of erythropoiesis: affects stem hematopoietic bone marrow cells contributing to their differentiation into recognizable precursors, stimulates maturation of erythroid precursor cells, proliferation of the maturing cells, delays apoptosis, accelerates synthesis of hemoglobin in erythroid cells and reticulocytes, takes part in formation of hemoglobin and erythrocyte cytoskeleton, increases blood flow in the erythropoietic bone marrow tissue and the input of reticulocytes into blood [29].

The main factor regulating the production of erythropoietin is hypoxia which leads to reduction of oxygenation in peritubular kidney cells secreting hemecontaining protein (cytochrome) binding an oxygen molecule. With lack of oxygenation, cytochrome activates the development of HIF-1 (a factor induced by hypoxia) which regulates erythropoietin synthesis and causes the activation of its production.

In the conditions of hypoxia, hypoxia sensitive ferments, namely phospholipase  $A_2$  enzymes are activated in the renal tubules, which leads to an increase in the level of the arachidonates that turn into endoperoxides in precursor cells with participation of cyclooxygenase. In addition, phospholipase  $A_2$  is responsible for production of prostaglandins ( $E_1$  and  $E_2$ ) in glomerular kidney cells that in their turn activate adenylatecyclase and cause an increase in cAMP concentration in peritubular kidney cells that synthesize erythropoietin. An important role in these biochemical reactions is played by calcium ions which stimulate the activity of phospholipase  $A_2$  and the formation of prostaglandin. Erythropoietin directly affects the metabolism of arachidonic acid carried out in normal erythroid precursor cells stimulating the absorption of calcium ions in the more differentiated stages, at the same time causing a significant increase of the concentration of free calcium. Thus, the interaction of erythropoietin with the cell membrane is a calcium-dependent process [30] (pic.).



**Рис.** Erythropoiesis stimulation

<i>Гипоксия</i>	<i>Hypoxia</i>
<i>Почка</i>	<i>Kidney</i>
<i>Фосфалипаза A<sub>2</sub></i>	<i>Phospholipase A<sub>2</sub></i>
<i>Арахидонатов</i>	<i>of arachidonates</i>
<i>ЦОГ</i>	<i>COX</i>
<i>Эндопероксидов</i>	<i>of endoperoxides</i>
<i>кп</i>	<i>Precursor cells</i>
<i>Эр</i>	<i>Erythrocytes</i>
<i>ЦАМФ</i>	<i>cAMP</i>
<i>Эритропоэтин</i>	<i>Erythropoietin</i>
<i>Кость</i>	<i>Bone</i>
<i>Костный мозг</i>	<i>Bone marrow</i>
<i>Созревание СК</i>	<i>Formation of free calcium</i>

In hypoxic conditions, the amount of blood-circulating erythropoietin increases approximately 1,000 times and reaches 5–30 u/ml [27, 28]. This process involves HIF-1 $\alpha$  [31]. One is to note that the majority of cells undergo erythroid differentiation when the erythropoietin concentration reaches 0.06 nM; one cell membrane can link with an average of 8 of its molecules [32].

Erythropoietin receptors are considered to belong to the family of cytokine receptors. Association of erythropoietin and its receptor launches various intracellular means providing for the function of erythroid row cells [33]. The receptors not only found in cells of hematopoietic lines, but also in other tissues like an epi-, peri- and myocardium, mesangial cells, muscular tissue fibroblasts and neurons [22, 32]. Data about the wide circulation of erythropoietin receptors in tissues allow to reasonable believe that deficiency in receptor-erythropoietin system can lead to various clinical manifestations [34].

### Use of erythropoietin drugs in pediatric surgery as a means of blood saving

In surgical practice, erythropoietin is mainly used as a complex therapy component prior to before self-administered blood collection [35–37]. Its application allows avoiding blood transfusion and reduces risk of infection in patients [38]. A lot of foreign (the USA, Switzerland, Australia, Italy) retrospective studies concerning the efficiency (for example, the reduction of hemotransfusion frequency during the operation) and safety of preoperative use of erythropoietin drugs in children of different ages (younger than 6 months and older than 8 years) for whom surgical correction of NsCS was planned.

In a number of studies, it was suggested to use erythropoietin preparations in a dose of 300–600 u/kg of body mass 1–3 times per week during 2–3 weeks together with iron preparations in the dose 2 of 10 mg/kg of body mass 2–3 times/day during the same period of time [35–38]. The treatment was accompanied by a weekly clinical blood test including dynamic hematocrit

control. For blood saving, the following "standard" procedures were used: acute normovolemic hemodilution of blood (Ht = 25%), controllable moderate arterial hypotension, and collection of the patient's own blood. These methods were applied both individually and in combination depending on their particular indications and contraindications. For preservation of stable intraoperative hemodynamics invasive monitoring was carried out. The children who took part in the studies were divided into 2 groups. The "standard" methods of blood saving were used in all the children; for the intervention group, a preoperative preparation was conducted with erythropoietin and iron preparations administration. No adverse effects or surgical complications were observed in the intervention group as a result of the therapy.

J.A. Ferron and J. Weinthal noted in the course of their study carried out on the basis of several leading US clinics, that in NsCS operations 93% of children of the control group and 57% of children who were administered erythropoietin and iron preparation needed hemotransfusion. In addition, in the latter, an increase of mean hemoglobin concentration (from 121 to 131 g/l) was observed, whereas in the control group there was no change of hemoglobin content [39].

The work by M.A. Helfaer et al. conducted in the chair for anesthesiology of John Hopkins medical university (Baltimore, Maryland, the USA), showed an increase in hematocrit from 34 to 43% within 3 weeks of therapy in the group of children who were administered erythropoietin, whereas in the control group this indicator remained constant (34%). In addition, in the course of surgical correction of NsCS hemotransfusion was conducted in 64% of children in the group administered erythropoietin/iron preparation compared to all the children in the control group. The authors of this study suggested that the reduction of frequency of hemotransfusion was related to higher hematocrit [40] and, therefore, to higher blood viscosity.

The study by J.G. Meara et al. conducted in a pediatric hospital (Melbourne, Australia) determined that of all the children who underwent endoscopic correction of NsCS only 50% of those administered erythropoietin and iron preparation needed hemotransfusion compared to 100% of the control group [41].

L. Meneghini et al. conducted a study on basis of the institute of hematology, immunology, and children's neurosurgery (Padua, Italy) that revealed that operative treatment of NsCS required hemotransfusion in 50% of the children who were administered erythropoietin and iron preparation and 70% of the children in the control group [42].

The study of R.G. Rohling et al. conducted in the department of maxillofacial surgery (University clinic of Zurich, Switzerland), the children who received erythropoietin and iron preparation in connection with blood saving methods needed hemotransfusion during the surgery in 13% of cases; in the control group, hemotransfusion was carried out in all the children [43].

In 2014, R.A. Vega et al. conducting their research based in a pediatric hospital (Richmond, USA) designed a protocol for reduction of the number of hemotransfusions in children who underwent surgical treatment of NsCS. The protocol encompassed 3 main components:

- Preoperative use of erythropoietin and iron drug;
- Use of Cell Saver during the operation;
- Admission of lower hemoglobin concentration (< 70 g/l) as an indication for hemotransfusion.

As a result of implementation of the protocol, an increase in hemoglobin concentration in patients was observed in the preoperative period. The comparison of results of operative treatment prior to and after the introduction of the protocol has shown that in 2012 compared to 2008 hemotransfusion was conducted in significantly fewer cases (56% of patients in 2012 versus 96% in 2008). It was accompanied by a decrease in surgical blood loss (212 ml in 2008 versus 114.5 ml in 2012) and operation duration (4 versus 2.8 hours, respectively). The mean duration of in-patient treatment was reduced (3.4 versus 2.6 days, respectively) [44].

In a number of other studies the authors have come to similar conclusions coming down to the fact that preoperative administration of erythropoietin with iron preparations combined with normovolemic hemodilution in children undergoing surgical correction of NsCS increases

hemoglobin concentration and therefore halves the demand for hemotransfusion [39-41]. Another typical observation is the reduction in in-patient treatment period of the patient, although this was not the key objective of the studies [42-44].

In Russia, the study of efficiency of human recombinant erythropoietin as a method of blood saving was the objective of a study conducted by V.V. Gromova et al. in 2010 on the basis of N.N. Burdenko research institute. Erythropoietin produced in Russia was used in neurosurgical patients with a background of preoperative anemia of various geneses with prognoses of massive blood loss during operative interventions. For stimulation of erythropoiesis and correction of anemia syndrome during 14 days before the neurosurgical intervention, 40,000 units of parenteral erythropoietin was administered every other day accompanied with iron preparations and vitamins. As a result, it was shown that erythropoietin therapy increases the preoperative hemoglobin level and hematocrit, which allows to use other means of blood saving during the operation (normovolemic hemodilution, mechanical reinfusion of washed erythrocytes) and decreases the volume donor blood used [45].

### **Safety of erythropoietin use in children**

Having analyzed scientific data on the use of erythropoietin drugs in adults for correction of anemia syndrome caused by chronic renal failure, A. Killian in 2002 mentioned the emergence of such undesirable phenomena as artery hypertension (in 24% of patients), headache (16%), painful joints (11%), nausea (11%), edema (9%), tiredness (9%), and diarrhea (9%). The profile of undesirable effects in children was similar to that of adults. Besides, single cases of stomachache, fever, infection of the upper airways, cough, pharyngitis, constipation [46] were observed.

A group of scientists in 2012 published a retrospective study of safe preoperative use of erythropoietin in surgical correction of craniosynostosis. In the course of this study, no complications were observed during in the use of erythropoietin in children who underwent surgical correction of the cranial vault from 2000 to 2008. The paper analyzes data of 369 children (with the mean age of  $0.86 \pm 1.1$  years). Only 31 participant (8.4%) of all had one or more postoperative complications not connected with the effects of erythropoietin. The insertion criteria comprised post-hemotransfusion reactions, pneumonias, infectious complications, deep vein thrombosis, stroke, embolism of the pulmonary artery, thrombosis of the sagittal sinus, aplasia of the red bone marrow, and myocardial infarction. The authors assessed the risk of development of thrombotic postoperative complications in children at less than 1%. These data allow assuming that preoperative use of erythropoietin in children who underwent surgical craniosynostosis correction does not lead to any significant negative reactions nor does it increase the risk of thrombogenesis [47].

In 1999, J.R. Brandt et al. conducted research on the basis of New Mexico pediatric hospital (the USA) hospital studying safe and efficient use of erythropoietin in patients with chronic renal failure. 44 patients aged from 4 months to 21 years took part in the study. In 30% of cases, an onset of arterial hypertension and iron deficiency was recorded. As a result, the authors expressed an assertion about safe and effective use of erythropoietin in children [48].

There have been numerous mentions of safe use of erythropoietin in children in Russian sources, especially lately [49,50]. For example, in 2009 a study of Y.V. Pilipenko dedicated to the use of recombinant human erythropoietin in prevention of anemia in premature babies with a very small and extremely low birth weight was published. The study was conducted in 61 children who were administered erythropoietin. As a result, it was demonstrated that the use of drug did not cause any adverse reactions or complications nor was accompanied in any case by the development of locals and an allergic reaction [50].

### **Conclusion**

Preoperative use of erythropoietin drugs with iron preparations in a combination with intraoperative normovolemic hemodilution is an effective and safe way of blood saving. Preoperative introduction of erythropoietin together with an iron preparation contributes to the reduction in the frequency of donor blood use in endoscopically assisted surgical correction NsCS in children. In addition, safety of using erythropoietin drugs in children was confirmed in a number of studies.

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### **Conflict of interest**

The authors declared they have no competing interests to disclose.

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