UNIVERSITY OF MEDICINE AND PHARMACY OF CRAIOVA
DOCTORAL SCHOOL

DOCTORAL THESIS

SUMMARY

ANEMIA OF PREMATURITY–ERYTHROPOIETIN VERSUS RED BLOOD CELL TRANSFUSIONS

SCIENTIFIC COORDINATOR:
Prof. Dr. FLORICA POPESCU

PhD STUDENT:
Dr. NICOLA C MIRELA ANIŞOARA (SIMINEL)

CRAIOVA

CONTENTS

1. STATE OF KNOWLEDGE.....1

2. PERSONAL CONTRIBUTIONS.....2

2.1 RESEARCH PURPOSE AND OBJECTIVES…… 2

2.2 MATERIALL AND METHODES……3

2.3 RESULTS AND DEBATES…….3

2.4 CONCLUSIONS..……..5

3. SELECTIV REFERENCES…….6

LIST OF ABBREVIATIONS

EPO	- erithopoietin
VG	- gestational age
Hb F	- Fetal Hemoglobin
Hb	- Hemoglobin
VLBW	- very low birth weight
ELBW	- extremely low birth weight
Ht	- Hematocrit
Zn PP	- zinc protoporphyrin
Zn PP/Hem	- zinc protoporfirin, Hem ratio
rHuEPO	- recombinant human erythropoietin
RBC	- red blood cell
US	- ultrasound

1. STATE OF KNOWLEDGE

Anemia of prematurity is a multifactorial anemia characterized by low levels of erythropoietin (Epo), iatrogenic blood loss, low circulating blood volume and lack of erythropoiesis [1].

It is a problem due to high incidence, associated symptoms and increased transfusion requirements [2]. It is a normochromic normocytic anemia hypo-regenerative that occurs between the 2nd and 6th weeks of age in premature infants with gestational age (GA) up to 35 weeks [2, 3].

It is an "exaggeration" of a physiological anemia due to decrease in red cell mass after birth, low life hemoglobin erythrocytes with fetal (Hb F) to 50% early rapid expansion of blood volume (10-15% per week) deficit vitamin E [2].

It is an "exaggeration" of a physiological anemia due to decrease in red cell mass after birth, low life hemoglobin erythrocytes with fetal (Hb F) to 50% early rapid expansion of blood volume (10-15% per week) deficit vitamin E [2].

The lower limit of hemoglobin (Hb) of a premature baby is lower than a term newborn and up to 6.5g / dl, clinically associated with decreased activity, growth failure, tachycardia, and tachypnea or sometimes without clinical expression [4].

Erythropoietin glycoprotein that stimulates division and maturation of erythroid cell lines is produced from the fetal life and it can be from 19-yh week in the umbilical cord [4].

Epo is synthesized in the kidney and in particular stimulated by the decrease of oxygen in renal flow. During fetal life Epo is produced in the liver reaching birth to occur mainly in the kidney [5].

During fetal development, circulating EPO concentrations increase from 4 mU/ml at 16 weeks to 40 mU/mL at term [6, 7, 8].

After birth, Epo levels of newborn babies at term decrease between 15-40 mU/mL immediately after birth to rise to reach the nadir between 4 and 6 weeks of life [9, 10]. Between 10 and 12 weeks old, adult concentration is reached (about15 mU/ml) [11].

Hepatocytes that synthesize Epo have low sensitivity to hypoxia such as premature baby is dependent on inefficient Epo production corresponding to a degree of anemia [12, 13]. It has long been considered that deficits of vitamin E, iron, folic acid and protein were most responsible for the early development of anemia [14].

Preterm birth deprives the fetus of a significant accumulation of iron in storage that occurs beyond 32- th. Week, total body iron deposits in tissues, Hb and serum ferritin are low in premature babies [15, 16, 7].

Without additional iron supply, very small birth weight and extremely small birth weight premature babies (VLBW, ELBW) can be negative iron balance in the first month of life with long-term consequences on the further development [18].

Iron deficiency affects perinatal growth, maturation and function of multiple organ systems including heart, skeletal muscle, gastrointestinal tract and brain [18,19, 20, 21].

Anemia of prematurity has been defined as low hematological constant levels of hemoglobin (Hb), hematocrit (Ht), serum iron, number of erythrocytes, the reticulocytes [2, 3,17].

Numerous studies have been done to determine the indices of erythropoiesis and stimulation of erythropoiesis, to estimate the iron stores and iron transport in conjunction with the clinic. [17, 18]

Thus, has been studied the cord Hb and cord serum ferritin correlated with neurological development of the child in the first year of life. Low levels of hemoglobin and serum ferritin cord were predictive of anemia of prematurity with neuro-psychomotor immediate and long effects [20, 21, 22, 23, 24].

Other studies have shown that free erythrocyte protoporphyrin, zinc protoporphyrin (PP Zn) and Zn PP, Hem ratio in perinatal hypoxia or if the iron supplement is sufficient for erythropoiesis [25, 26, 27].

Serum iron and serum transferrin saturation does not accurately reflect iron transport compartment in the perinatal period. The management aims of anemia of prematurity is to maintain as intact red blood cells of premature baby and stimulate the appropriate production of erythrocytes [19, 20, 21].

Delayed clamping of the umbilical cord and to avoid excess blood samples for diagnosis are ways to reduce blood loss [28].

After Cochrane Central Library review, early in the 1990s the treatment of anemia of extremely low (ELBW) premature babies (under 1000g) and very low birth weight (VLBW) between 1000-1500 g consist in multiple blood transfusions [29, 30, 31].

Between 1990 and 2013, according to The Register of Controlled Trials were performed 59 studies in 20 countries on the treatment of anemia including over 2,000 premature [29, 30].

These studies demonstrated the efficacy and safety of early or late initiation of therapy with EPO while adequate iron supplementation, vitamin E and folic acid, in the prevention and treatment of anemia of prematurity and reduce the number of transfusions [31, 32, 33, 34, 35, 36].

2. PERSONAL RESEARCH

Thesis aims is to analyze issues related to the treatment of anemia of prematurity with recombinant human erythropoietin rHuEPO versus red blood cell transfusions. It were investigated clinically and laboratory (dynamic values of parameters Hb, Ht, HEM, Astrup), two comparable groups of preterm low birth weight (below 1500g).

Statistical results show that to achieve similar values of hematological parameters, rHuEpo treated group had a better evolution in order to reduce the incidence and severity of intraventricular hemorrhage, retinopathy of prematurity reduce the incidence and severity, extension of time of occurrence of the nadir level of hemoglobin, reducing the need for red blood cell transfusions (number decreased significantly).

The originality of the study is that there was noted a temporal relationship between red blood cell transfusion and the increased incidence of intraventricular hemorrhage or increased the severity of existing ones.

2.1 PURPOSE OF THE PAPER

The purpose of this paper is to analyze the efficacy and safety of anemia of prematurity treatment with rHuEPO versus red blood cell transfusions, transfusion volume, management and statistically significant correlations between the pathology associated with prematurity in a number of 127 premature infants admitted to the

Neonatal Clinical County Hospital Emergency Craiova, for a period of three years (2007-2010)

1.2.MATERIAL AND METHODS

The study was designed as a prospective comparative one for a period of three years from 2007 to 2010 including 127 preterm babies with ages: 24 to 31 weeks and weighing between 690 -1500g, divided into two groups: one treated with EPO and the second treated with red blood cell transfusions, as follows:

The first group treated with EPO, composed of two subgroups: subgroup E1 of 6 preterm extremely low birth weight between 690-1000g (ELBW) and gestational age (GA) 24-26 weeks subgroup E2 format of 56 premature infants with very low birth weight (VLBW) between 1000-1500g and gestational age (GA) 27 to 31 weeks.

The latter group treated with red blood cell transfusion (RBC) consists of 2 subgroups: T1 subgroup of 7 ELBW premature babies weight between 760-1000g and gestational age between 25-27 weeks and subgroup T2 VLBW premature babies weight between 1000-1500g and gestational age (GA) 27 to 31 weeks.

For each included premature baby was taken the informed consent of the parents, being respected the ethical medical guidelines for research including human subjects.

Criteria for inclusion in the study:
- Premature infants with gestational age below 32 weeks
- Premature infants weighing less than 1500g.

Exclusion criteria from the study:
- hemolytic anemia,
- hemorrhagic disease of the newborn,
- congenital anemia,
- cyanotic heart malformation,
- birth defects
- intraventricular hemorrhage greater than grade 3,
- sepsis,
- infections of the TORCH,
- necrotizing enterocolitis (NEC)

1.3. RESULTS AND DISCUSSION

The doctoral thesis by the research conducted purpose to examine issues related to the treatment of anemia of prematurity with rHuEPO versus red blood cell transfusions. To research comparative evolution of associated pathology with prematurity: respiratory distress syndrome, pulmonary bronhodisplasy, retinopathy of prematurity, intraventricular hemorrhage, periventricular leukomalacia, necrotizing enterocolitis and decrease transfusions and their default risks and complications. Such were investigated clinically, laboratory (dynamic values of parameter Hb, Ht, Astrup) and imaging: head ultrasound in dynamic day 1, 3, 7, 14, 30, and at discharge, radiographic exams, the two comparable groups preterm with very low birth weight (sub1500g), one receiving treatment with rHuEPO the second receiving red blood cell transfusions (RBC).

Statistical results showed that, to achieve similar values of hematological parameters, rHuEpo treated group evolution was better in order to reduce the incidence

and severity of intraventricular hemorrhage, a reduction in the incidence of retinopathy of prematurity and severity, prolongation of occurrence of the nadir hemoglobin, reduce the need for transfusions (number decreased significantly).

The clinical symptoms of anemia of prematurity were associated: pale skin (34%), apnea (14%), tachycardia, murmurs, weight loss (30%).

Regarding the number of transfusions received by the preterm infants in the two groups, there is a highly significant difference ($p = 9.39 \times 10^{-18} \approx 0 < 0.001$) between the two groups increased number of transfusions at group transfused comparative with the rHuEpo group. In the group treated with rHu Epo the 38 (61.29%) of premature there has been no transfusion after starting treatment with rHuEPO (after day 8 a), in contrast, between the subjects from the other lot, a number of 41 (63. 08%) received two transfusions, 12 (18.46%) with one transfusion, other 12 (18.46%) benefited from three or more transfusions.

Of the latter two (3.07%) of premature babies required 5 transfusions, 4 (6.15%) preemies required four transfusions and 6 (9.23%), premature infants required a number of 3 transfusions.

Erythropoietin treatment protocol included late administration after the 8th day, thus in the first week the subgroup E1 (ELBW) a number of 6 premature, 4 of them (6.45%) required two transfusions and two one transfusion, in the other 56 preterm babies from subgroup E2 (VLBW), 18 (29%) received a transfusion of packed red blood cells.

In total, the group treated with rHuEPO, 24 (38.71%) preterm babies received RBC transfusions the rest have not been transfused in the first week.

A similar situation is found in the group treated with RBC transfusions in the first week, 23 (35.38%) received one transfusion of premature infants and the other 4 (6.15%) of subgroup T1 (ELBW) received two RBC transfusions the first week

After the study starts, those (RBC transfused group) that received one or two first week transfusions, necessitated another one or two or even three RBC transfusions.

In all of transfused group, 12 (18.46%) premature infants receiving one transfusion, 41 (63.08%) received two RBC transfusions and 12 (18.46%) had received three or more transfusions within 5.

Clinics in transfused group revealed the decrease of apnea attacks after transfusion [37].

Head ultrasound (head US) is a diagnostic method available, portable, inexpensive, non-invasive, which can reveal changes that are predisposed small premature infants: intraventricular hemorrhage or periventricular leukomalacia.

The ultrasound examination revealed changes significantly higher in neonates treated only by RBC transfusions than in those treated with rHuEPO. Scans performed in the first week showed a balanced between the two groups therefore, in the group treated with rHuEPO, 22 (35.48%) had intraventricular hemorrhage grade I and 16 (25.80%) grade II hemorrhage versus transfused group which have been identified intraventricular hemorrhage at 25 (38.46%) grade I and 15 (23.07%) grade II intraventricular hemorrhage.

In the group treated with rHuEPO, ultrasound performed on day 14 revealed at 38 (61.29%) of premature babies intraventricular hemorrhage images: 27 (43.55%) grade I and 11 (17.74%) grade II, compared to 52 (80%) of the other group RBC transfused at which the intraventricular hemorrhage appears in varying degrees: 29 (46.77%) grade I, 12 (35.48%) grade II, 9 (14.51%) degree III and 3 (4.83%) grade IV. Between these the 3 preterm with hemorrhage grade IV were premature with extremely low birth weight

(ELBW) and 4 patients with grade III hemorrhage and the rest of 5 were premature infants weighing between 1000-1500g (VLBW).

At discharge in the RBC transfused group, 9 (13.85%) of cases had ventriculomegaly versus 3 (4.84%) in the EPO group and hydrocephalus was observed in 3 (4.62%) in the group receiving RBC transfusions and none in the EPO group.

The cases with ventriculomegaly had grade III intraventricular hemorrhage which evolved to ventriculomegaly, and cases with grade IV intraventricular hemorrhage developed hydrocephalus.

The particularity of the study is that there was a temporal relationship between RBC transfusion and appearing of intraventricular hemorrhage or worsening a preexistent one to a severe form. The phlebotomy blood loss by phlebotomy was calculated at 7ml/premature/week.

RBC transfused volume was small (10 ml / kg) and a number of 12 preterm of transfused group received more than 3 transfusions (all infants weighing less than 1000g).

Since intraventricular hemorrhages occurred immediately after transfusion of RBC transfusions they seem to be a risk factor in premature infants developing severe intraventricular hemorrhage severe. The results are compared to other studies in the literature that show the importance of rHuEPO in reducing the risk of intraventricular hemorrhage [38, 39].

Among the 12 children who received more than 3 RBC transfusions (within 14 days) we found at head ultrasound at 14-th day that 9 of them have progressed to hemorrhage grade III and three of them to grade IV hemorrhage.

A significant association for the occurrence of bleeding in <48h after transfusion or appearance of early signs of necrotizing enterocolitis suggests that oscillatory changes of cerebral blood flow or circulatory overload in defferent territories mainly brain and mesenteric circulation should be closely linked to RBC transfusions [40, 41, 42].

Moreover the group treated with rHuEPO ultrasound revealed no serious elements regarding, hemorrhages suggesting a protective effect of rHuEPO on the premature brain. Between the 62 children, 27 (43.55%) had grade I intraventricular hemorrhage and 11 (17.74%) of the grade II intraventricular hemorrhage while only 3 (4.84%) cases were developed and third-degree aggravated the rest being reabsorbed into the restitutio ad integrum.

The total amount of RBC transfused was 1995ml, with an average volume of 30.69 ml/ child of red blood cells.

A larger volume transfusion adversely affects premature high not only on the brain but also in the production of chronic lung disease.

2.4.CONCLUSIONS

1. The two groups reached at the same values of hematocrit and hemoglobin at discharge in terms of eliminating the need for transfusion of premature infants treated group rEPO.

2. A temporal relationship has been observed between RBC transfusion and appearing severe intraventricular hemorrhage and/or developed severe grades of preexistent ones.

3. There is unclear if a causal relationship exist between RBC transfusion and hemorrhage or a co-variable one.

4. In the same time, is observed the protective effect of rHuEPO (associated with iron, folic acid and vitamin E), in premature infants that head ultrasound did not revealed images of different sever degree of intraventricular hemorrhage.

5. Multiple RBC transfusions in premature infants with low weight have a significant risk for severe intraventricular hemorrhage grade 3 and 4 including the evolving hydrocephalus and long-term neurologic prognosis.

6. It can be limited the number of RBC transfusions by limiting blood loss thru phlebotomy by collection and analysis using the micromethod or microvacutainere.

7. Limiting the number of red blood cell transfusions lead to limitation the risks of infectious agents: human immunodeficiency virus, hepatitis B virus, hepatitis C, syphilis, T-limfotrofic virus (HTLV) I/II, cytomegalovirus, possible transmitted through transfusions.

3. SELECTIV REFERENCES

1. De Pedro A. De Alarcon, Eric J Werner, Anemia of prematurity, Neonatal Hematology 2005 pag. 58-59
2. Spitzer A, Anemia of prematurity, Intensive Care of Fetus and Neonatal Second Edition 2005 pag. 1286
3. Cloherty, John P.; Eichenwald, Eric C.; Stark, Ann R. Manual of Neonatal Care, 6th Edition, 2008
4. H. William Taeusch, Roberta A. Ballard, Christine A. Gleason,Mary Ellen: Avery's Diseases of the Newborn 8-th ed, 2005
5. MesserJ, Donato L, Astruc D, Matis J., *Pediatrics* 1993, pag. 92 519-523
6. Widness J.A., Susa J.B., Garcia J.F., et al.: Increased erythropoiesis and elevated erythropoietin in infants born to diabetic mothers and in hyperinsulinemic rhesus fetuses. J Clin Invest. 67:637-642 1981
7. Ireland R, Abbas A, Thilaganathan B, *et al.:* Fetal and maternaI erythropoietin levels in normal pregnancy. *Fetal Diagn Ther* 7: 21-25, 1992
8. Ruth V. Widness JA, Clermonts, Raivio, KO: Postnatal changes in serum immunoreactive erythropoietin in relatin to hipoxie before and after birth, J Pediatr 116:950-944, 1990
9. Widness J.A., Susa J.B., Garcia J.F., et al: Increased immunoreactivity of erythropoietin in cord serum after labor , Am J. Obstet Gynecol, 148: 194-197,1984
10. Brown MS, Phibbs RH, Garcia JF, Dallman PR. Postnatal changes in erythropoietin levels in untransfused premature infants. J Pediatr. 1983 Oct;103(4):612–617
11. Kling PJ, Schmidt, RL, Roberts RA, Widness JA: Serrum erythropoietin levels during infancy associations with erythropoiesis, J Petiatr 1996; 128:791-6
12. Malcom L Chiswick, Protective effect of vitamin E(Dl-alfa-tocopherol) against intraventricular haemorrhage in premature babies, British Mediacal Journal, Volume 287, 1993
13. Lackmann GM, Schnieder C, Bohner J. Gestational age-dependent reference values for iron and selected proteins of iron metabolism in serum of premature human neonates. *Biol Neonate.* 1998;74:208–13
14. Malcom L Chiswick: Protective effect of vitamin E(Dl-alfa-tocopherol) against intraventricular haemorrhage in premature babies, British Mediacal Journal, Volume 287, 1993
15. Armony-Sivan R, Eidelman AI, Lanir A, Sredni D, Yehuda S: Iron status and neurobehavioral development of premature infants. *J Perinatol.* 2004;24:757–62
16. Singla PN, Gupta VK, Agarwal KN. :Storage iron in human foetal organs. *Acta Paediatr Scand.* 1985;74:701–6

17. Janet M Rennie, Rennie & Roberton's Textbook of Neonatology Fifth Edition, 2012

18. Genen LH, Klenoff H: Iron supplementation for erythropoietin-treated preterm infants (Protocol). In: The Cochrane Database of Systematic Reviews, Issue 1, 2001

19. Deugnier Y, Brissot P, Loreal O: Iron and the liver: *Update 2008*

20. Siimes AS, Siimes MA: Changes in the concentration of ferritin in the serum during fetal life in singletons and twins. *Early Hum Dev.* 1986;13:47–52

21. Tamura T, Goldenberg RL, Hou J, Johnston KE, Cliver SP, Ramey SL, et al.: Cord serum ferritin concentrations and mental and psychomotor development of children at five years of age. *J Pediatr.* 2002;140:165–70

22. Georgieff MK, Wewerka SW, Nelson CA, Deregnier RA. Iron status at 9 months of infants with low iron stores at birth. *J Pediatr.* 2002;141:405–9

23. Beard J, De Regnier RA, Shaw M, et al. Diagnosis of iron deficiency in infancy. *Lab Med.* in press

24. Lozoff B, Beard J, Connor J, Barbara F, Georgieff M, Schallert T. Long-lasting neural and behavioral effects of iron deficiency in infancy. *Nutr Rev.* 2006;64:S34–91

25. Sheldon RA, Almli L, Ferriero DM (2002) Copper/zinc superoxide dismutase transgenic brain in neonatal hypoxia-ischemia. Methods Enzymol 353:389-397

26. Juul SE, Zerzan JC, Strandjord TP, Woodrum DE. Zinc protoporphyrin/heme as an indicator of iron status in NICU patients. *J Pediatr.* 2003;142:273–8

27. Lott DG, Zimmerman MB, Labbe RF, Kling PJ, Widness JA. Erythrocyte zinc protoporphyrin is elevated with prematurity and fetal hypoxemia. *Pediatrics.* 2005;116:414–22

28. Lainez Villabona B[1], Bergel Ayllon E, Cafferata Thompson ML, Belizán Chiesa JM.: Early or late umbilical cord clamping? A systematic review of the literature, An Pediatr (Barc). 2005 Jul;63(1):14-21

29. Arne Ohlsson, Sanjay M Aher: Early erythropoietin for preventing red blood cell transfusion in preterm and/or low birth weight infants, Cochrane Database of Systematic Reviews 2014, Issue 4. Art. No.: CD004863. DOI: 10.1002/14651858.CD004863.pub4

30. Aher S, Ohlsson A, Late erithropoietin for preventing red blood cell transfusion in preterm and/or low birth weith infants(Review), The Cohrane Library, 2014

31. Isabelle Von Kohorn, Richard A Ehrenkranz: Anemia in the preterm infant: Erythropoietin versuserithrocyte transfusion-It's not that simple, Clin Perinatol. 2009; 36(1):111-123.

32. Kotto-Kome AC, Garcia MG, Calhoun DA, Christensen RD. Effect of beginning recombinant erythropoietin treatment within the first week of life, among very-low-birth-weight neonates, on "early" and "late" erythrocyte transfusions: a meta-analysis. *Journal of Perinatology* 2004;24:24-9

33. Ohlsson A, Aher SM. Early erythropoietin for preventing red blood cell transfusion in preterm and/or low birth weight infants. Cochrane Database of Systematic Reviews 2006, Issue 3. Art. No.: CD004863. DOI: 10.1002/14651858.CD004863.pub2.

34. Ohlsson A, Aher SM. Early erythropoietin for preventing red blood cell transfusion in preterm and/or low birth weight infants. Cochrane Database of Systematic Reviews 2012, Issue 9. Art. No.: CD004863. DOI: 10.1002/14651858.CD004863.pub3.

35. Ohls RK. Erythropoietin treatment in extremely low birth weight infants: blood in versus blood out. Journal of Pediatrics 2002;141(1):3-6

36. Maier RF, Sonntag J, Walka MM, Liu G, Metze BC, Obladen M. Changing practices of red blood cell transfusions in infants with birth weights less than 1000 g. Journal of Pediatrics 2000;136(2):220-4.

37. Kelley Zagol, Douglas E. Lake, Brooke Vergales, Marion E. Moorman, Alix Paget-Brown, Hoshik Lee, Craig G. Rusin, John B. Delos, Matthew T. Clark, J. Randall Moorman, and John Kattwinkel: Anemia, Apnea of Prematurity, and Blood Transfusions, (J Pediatr 2012;161:417-21).

38. Baer VL, Lambert DK, Henry E, Snow GL, Christensen RD. Red blood cell transfusion of preterm neonates with grade 1 intraventricular hemorrhage is associated with extension to a grade 3 or 4 hemorrhage. Transfusion 2011;51(9):1933-9.

39. Juul S. Erythropoietin in the central nervous system, and its use to prevent hypoxic-ischemic brain damage. Acta Paediatrica Supplement 2002;91(438):36-42

40. Ledbetter DJ, Juul SE (2000) Erythropoietin and the incidence of necrotizing enterocolitis in infants with very low birth weight. J Pediatr Surg 35:178-181
41. Blau J, Calo JM, Dozor D, Sutton M, Alpan G, La Gamma EF. Transfusion-related acute gut injury: necrotizing enterocolitis in very low birth weight neonates after packed red blood cell transfusion. Journal of Pediatrics 2011;158(3):403-9.
42. Kirpalani H, Zupancic JA. Do transfusions cause necrotizing enterocolitis? The complementary role of randomized trials and observational studies. Seminars in Perinatology 2012;36(4):269-76.