“VICTOR BABES” UNIVERSITY OF MEDICINE AND PHARMACY TIMISOARA

FACULTY OF GENERAL MEDICINE

Department XII – Obstetrics and Gynecology

BILAV OANA CRISTINA

DOCTORAL THESIS

IMPLICATIONS OF USING ERYTHROPOIETIN IN PERINATAL PATHOLOGY

Scientific Coordinator

PROF. UNIV. DR. HABIL. BOIA MARIOARA

T i m i s o a r a

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TABLE OF CONTENTS

STUDY 1: THE ROLE OF ERYTHROPOIETIN IN PREVENTING ANEMIA IN THE PREMATURE NEONATE.

CONTEXT

Anemia of Prematurity (AOP) is a multifactorial condition that typically arises between the second and sixth weeks of life, influenced by factors such as diminished erythropoietin (EPO) levels, iatrogenic blood loss, and reduced red blood cell survival. It predominantly affects preterm neonates under 35 weeks of gestation, with the liver's sluggish response to anemia and the frequent laboratory tests exacerbating the condition. This high incidence of AOP, coupled with its symptomatology and the increased need for transfusions, underscores the challenges it poses in pediatric healthcare, prompting a reevaluation of current treatment protocols to improve outcomes.

Over the last forty years, recombinant human EPO (rhEPO) has emerged as a pivotal treatment for AOP, demonstrating its ability to stimulate erythropoiesis and decrease the necessity for red blood cell transfusions. Despite extensive research into rhEPO's efficacy and potential side effects, debates continue regarding the optimal timing for its administration, with evidence suggesting varied effects on increasing reticulocyte counts and hemoglobin levels. The utilization of rhEPO, particularly in preterm neonates, has shown promising results in reducing the frequency and dosage of transfusions required, marking a significant shift in the management of AOP.

Given these findings, a non-randomized controlled trial aimed at assessing the impact of early rhEPO administration on the incidence and severity of AOP, as well as the necessity for RBC transfusions, is crucial. By examining the effects of rhEPO within the first week of life on the incidence rate of AOP at 21 days, varying levels of AOP severity, and subsequent impacts on hemoglobin, hematocrit, and serum EPO levels at three weeks, this study seeks to illuminate the potential of rhEPO as a neuroprotective agent in neonatal care. This investigation not only contributes to our understanding of AOP management but also opens avenues for future research focused on refining treatment protocols to enhance neonatal health outcomes.

SUMMARY OF FINDINGS

This study explores the incidence of Anemia of Prematurity (AOP) among neonates in relation to several biochemical markers and treatment interventions. It was found that neonates with pathological levels of lactate dehydrogenase (LDH), prothrombin time (PT), and serum erythropoietin (EPO) on the first day of life exhibited significantly higher rates of AOP. Conversely, neonates with abnormal activated partial thromboplastin time (aPTT) levels displayed a lower incidence of AOP compared to those with normal levels. Interestingly, the administration of iron between the 7th to 21st days of life was associated with an increased incidence of AOP, whereas early administration of EPO appeared to reduce the AOP incidence.

The study further delved into the relationship between these factors and the necessity for red blood cell transfusions within the first 21 days of life. It was observed that a higher percentage of male neonates required transfusions compared to female neonates. The need for transfusions decreased with increased gestational age (GA) and birth weight (BW). Neonates with pathological PT levels were more likely to require transfusions than those with normal levels, a trend that was not observed in neonates with abnormal aPTT levels. Similar to the findings on AOP incidence, iron supplementation was linked to a higher transfusion rate, while EPO administration within the first week was associated with a reduced transfusion requirement.

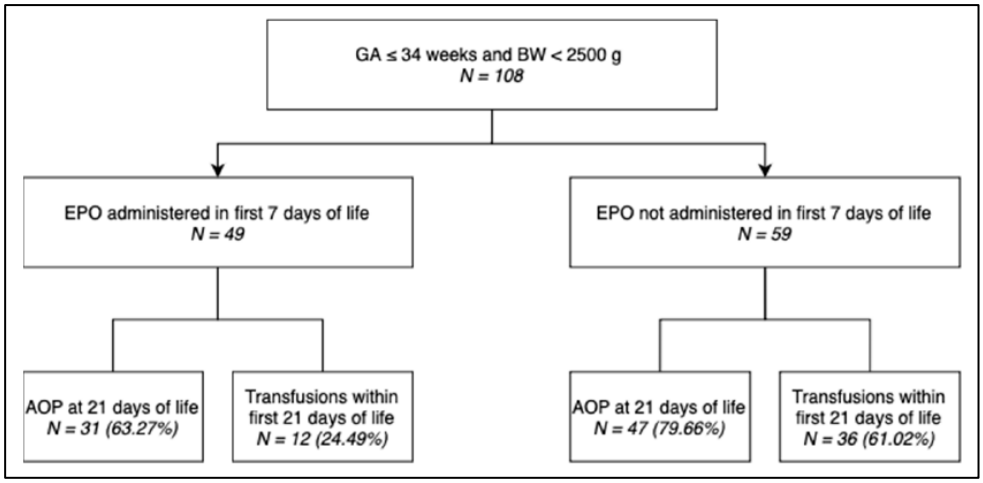
A detailed univariate analysis revealed no significant baseline differences between neonates who did and did not receive EPO treatment within the first 7 days, except for serum EPO levels. This analysis aimed to identify any disparities that could influence the study's outcomes. Regarding risk factors for AOP and transfusions, gestational age, birth weight,

hemoglobin levels, hematocrit, and red blood cell count emerged as protective factors against AOP, while iron administration was identified as a risk factor. Conversely, serum EPO levels were flagged as a significant risk factor for requiring transfusions within the first 21 days.

Multivariate analysis confirmed that iron administration between the 7th and 21st days significantly increased the risk of developing AOP, underscoring the complexity of managing anemia in premature neonates. This finding was consistent across various analyses, suggesting a nuanced relationship between iron supplementation and AOP development. Furthermore, the study investigated associations with specific AOP stages and transfusion requirements, highlighting the influence of early EPO administration on reducing moderate AOP incidence.

The study's findings suggest a delicate balance in the management of anemia in premature neonates, indicating that while certain interventions can mitigate the risk of AOP and transfusion requirements, others may inadvertently increase such risks. These insights into the multifaceted nature of neonatal care underscore the importance of tailored medical approaches based on individual biochemical markers and early life interventions.

Figure 1 – Distribution of neonates included in the study.



CONCLUSIONS

In this study, rhEPO treatment within the first 7 days of life was confirmed to reducemoderate AOP incidence and increase Hb, HCT, and serum EPO levels at 21 days of life.Additionally, rhEPO therapy has been significantly associated with reduced incidence oftransfusions within the first 21 days of life. These results underline the multiple benefits ofrhEPO treatment in preterm neonates.

STUDY 2: THE EFFECTS OF IRON ADMINISTRATION DURING THE 7TH AND 21ST DAY OF LIFE IN PREMATURE NEWBORNS.

CONTEXT

Iron plays a critical role in the development and functioning of various organs, particularly in newborns, where it supports neurological development through myelination, neurotransmitter synthesis, and energy metabolite transport. However, iron deficiency (ID) remains a global nutritional disorder, leading to significant developmental challenges, especially in the brain's areas responsible for memory, learning, and motor activities. The allocation of iron primarily to erythropoiesis during fetal and newborn development, often due to maternal diabetes and placental dysfunction, exacerbates the risk of ID, impacting the hippocampus and other critical regions involved in cognitive processes.

The administration of iron supplements in newborns has been recognized for its protective effects against ID and anemia, although the long-term effects of iron overload remain under-researched. While most studies suggest no significant adverse effects of iron supplementation on neurological development, concerns have been raised regarding potential side effects, including gastrointestinal issues, growth delays, and respiratory infections. Yet, there's no evidence linking iron supplementation to serious newborn diseases like periventricular leukomalacia, pulmonary disease, necrotizing enterocolitis, or retinopathy of prematurity, nor an increased risk of anemia of prematurity (AOP).

This study aims to investigate the efficacy and potential side effects of iron supplementation between the 7th and 21st day of life in preterm newborns, analyzing how this intervention and other perinatal factors affect outcomes like AOP severity and serum ferritin and iron levels. The need for further research into optimal dosing and delivery strategies is highlighted, given the varying supplementation approaches and the dearth of long-term studies addressing the consequences of iron overload. This underscores the importance of finding a balance in iron administration to support newborn development while minimizing potential risks.

RESULTS

The study assessed the impact of iron supplementation on anemia of prematurity (AOP) among 108 premature newborns with gestational ages (GA) ranging from 24 to 34 weeks and birth weights (BW) from 650 to 2500 grams. It specifically looked at those who received iron between the 7th and 21st day of life compared to those who did not. The findings indicated a significant difference in AOP incidence, abnormal serum ferritin levels, and abnormal serum iron levels between the two groups, with higher rates observed in the group that received iron supplementation.

A detailed analysis of the distribution of AOP and abnormal serum levels across various baseline characteristics revealed a broader impact. The study noted that 80% of newborns who received iron developed AOP, a stark contrast to the 62.5% in the control group. This trend was consistent across other metrics, with abnormal serum ferritin and iron levels also more prevalent in the iron-supplemented group. These outcomes suggest a complex relationship between iron supplementation and neonatal health outcomes, particularly concerning AOP.

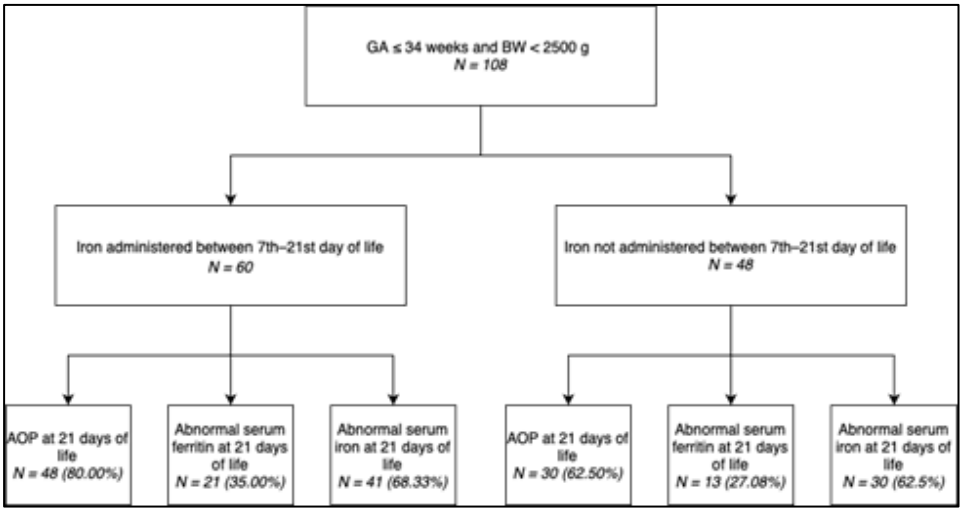
The investigation into baseline characteristics and the comparison between newborns who received iron supplementation and those who did not revealed no significant differences in most parameters, including sex, GA, BW, Apgar scores, and day-1 hemoglobin, hematocrit, red blood cell count, activated partial thromboplastin time, prothrombin time, lactate dehydrogenase, and serum erythropoietin levels. However, a notable exception was found in day-1 serum erythropoietin levels, which were significantly higher in the intervention group. This underscores the importance of considering the multifaceted role of erythropoietin in neonatal health and development.

Univariate logistic regression analysis further elucidated the factors influencing AOP incidence, identifying GA, BW, day-1 hemoglobin and hematocrit levels, and day-1 red blood cell count as protective against AOP. Conversely, iron supplementation was highlighted as a risk factor for developing AOP, suggesting that while iron is crucial for neonatal development, its administration must be carefully managed to avoid adverse outcomes such as AOP.

Multivariate logistic regression analysis refined these insights, pinpointing iron administration during the critical 7th to 21st day of life window as a significant risk factor for AOP, with a more than twofold increase in risk. This analysis reaffirms the complex nature of iron's role in neonatal care, emphasizing the need for judicious use of supplementation to balance the benefits of preventing iron deficiency against the risks of contributing to AOP development.

Finally, the study's ANCOVA analysis on serum ferritin and iron levels post-iron administration revealed no significant correlations, indicating that while iron supplementation within the specified timeframe influences AOP incidence, it does not directly correlate with changes in serum ferritin or iron levels at 21 days of life. This suggests that the mechanisms by which iron supplementation affects neonatal outcomes may involve more intricate biochemical pathways or interactions than previously understood, highlighting the need for further research to optimize iron supplementation strategies in premature newborns.

Figure 2 – Distribution of newborns by iron administration status and further stratified by frequency of AOP and abnormal serum ferritin and iron levels.



CONCLUSIONS

This study confirms the limited effectiveness of early iron supplementation in pre-term newborns between the 7th and 21st days of life in reducing ID risk and stabilizing or increasing serum iron and ferritin and presents a novel finding, namely the increased risk of developing AOP at 21 days of life. Therefore, it is recommended that subsequent re-search on the optimal iron dosing in order to achieve a better benefit/risk ratio, as well as on alternative nutrient supplementation strategies that can better prevent ID while pre-senting fewer risks to newborns.

STUDY 3: IMPACT OF EARLY ERYTHROPOIETIN THERAPY ON NEURODEVELOPMENT AND PREVENTION OF INTRAVENTRICULAR HEMORRHAGE AND HYPOXIC-ISCHEMIC ENCEPHALOPATHY IN NEWBORNS.

CONTEXT

Erythropoietin (EPO), traditionally known for its role in stimulating red blood cell production, has gained interest for its potential neuroprotective effects in neonatal medicine. The production of EPO is a response mechanism to hypoxia, regulating not only erythropoiesis but also playing a significant role in the brain's response to injury. This dual function of EPO positions it as a critical agent in addressing neonatal brain injuries, which are particularly concerning due to the vulnerability of newborns to hypoxia-ischemia, inflammation, and hemorrhage, and the associated risks of intraventricular hemorrhage (IVH) and hypoxic-ischemic encephalopathy (HIE) prevalent in preterm infants.

The neurological development of newborns, especially preterm ones, faces significant risks with a high incidence of neurodevelopmental impairments and complications like IVH and HIE. The search for effective neuroprotective strategies has highlighted the potential of EPO due to its roles beyond erythropoiesis, including neuroprotection, angiogenesis, and anti-inflammation. Studies, including landmark research from 2016, suggest that high-dose EPO administration in preterm infants can lead to improved neurodevelopmental outcomes, a finding supported by animal models demonstrating EPO's capacity to mitigate brain injury.

However, the application of EPO in neonatal care brings challenges, including determining the optimal dosage, timing, and therapy duration, alongside concerns over potential long-term effects such as retinopathy. With ongoing research into EPO's effects on synaptic plasticity, neurogenesis, and myelination, this study aims to further investigate EPO's impact on neonatal neurological development. The goal is to establish the efficacy of early EPO administration on improving neurological outcomes in newborns at risk for neurodevelopmental disorders, evaluate the short-term outcomes, and assess the safety profile of EPO therapy, thereby contributing to enhanced neonatal neuroprotective care standards.

RESULTS

The study meticulously analyzed background characteristics among neonates, revealing no significant differences in critical indicators such as gestational age, birth weight, sex distribution, and APGAR scores between the EPO and No EPO groups. This equivalence in baseline conditions between the two cohorts allowed for a focused examination of the erythropoietin (EPO)'s impact, setting a solid foundation for the subsequent analysis of outcomes. The laboratorial investigations further enriched the study by identifying significant disparities in early postnatal biochemical markers. Notably, initial lactate dehydrogenase (LDH) levels were higher in the EPO-treated group, a difference that intriguingly inverted by the second week, suggesting a potential early impact of EPO on neonatal metabolic processes. Additionally, a range of other metabolic and inflammatory markers, including blood glucose, urea, creatinine, and C-reactive protein, showed lower median values in the EPO group, indicating the hormone's broader systemic effects beyond erythropoiesis.

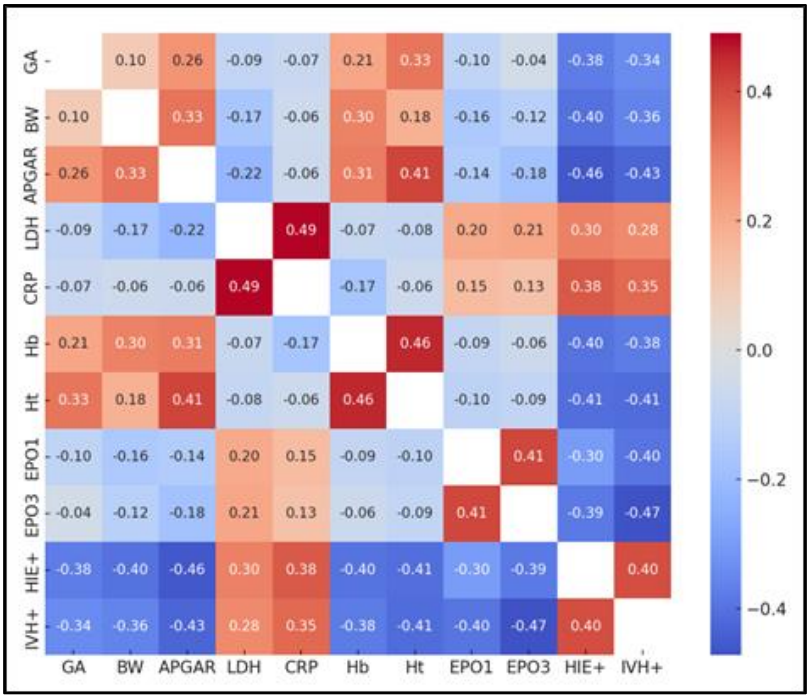
The study's outcome analysis provided insightful findings into the clinical implications of EPO administration. Initially, the incidence of ventriculomegaly and intraventricular hemorrhage (IVH) showed no significant differences between groups, suggesting a neutral effect of EPO on these conditions in the immediate postnatal period. However, a marked difference emerged by the fourth week, with the EPO group exhibiting significantly lower incidences of moderate and severe hypoxic-ischemic encephalopathy (HIE) and a notable reduction in severe IVH cases. This shift underscores the potential of EPO therapy in mitigating long-term neurodevelopmental complications in neonates, highlighting its role in

enhancing neuroprotective strategies within neonatal care. Moreover, the occurrence of bradycardia was significantly higher in the EPO-treated neonates, signaling a need for vigilant monitoring for potential adverse effects associated with EPO administration.

Further analysis through logistic regression underscored the protective role of early EPO administration against the development of IVH, evidencing a 41% reduction in the odds of this condition in the EPO group. This significant finding suggests that timely EPO therapy could serve as a crucial intervention for reducing the risk of IVH, a common and severe complication in preterm neonates. The analysis also spotlighted gestational age and birth weight as significant predictors of IVH risk, emphasizing the increased vulnerability of extremely and very preterm infants to this condition. These insights are pivotal for tailoring neonatal care strategies to mitigate IVH risk effectively.

The multiple linear regression analysis extended the study's scope by examining the severity of HIE, revealing that early EPO administration significantly diminished HIE severity. This finding corroborates the neuroprotective potential of EPO, suggesting its efficacy in not only reducing the incidence but also the severity of neurodevelopmental impairments. The analysis also highlighted the critical influence of gestational age and birth weight on HIE severity, with extremely and very preterm gestations, as well as lower birth weights, associated with increased severity of HIE. These results further delineate the risk profile for HIE, underscoring the importance of targeted interventions in these high-risk groups.

Figure 3 – Correlation analysis.



CONCLUSIONS

In conclusion, there is evidence that early EPO administration within the first 48 hours of birth plays a critical role in mitigating the risks and severity of neonatal compli-cations such as IVH and HIE. The substantial reduction in the occurrence and severity of these conditions, indicated by significant statistical measures, underlines the therapeutic potential of EPO in neonatal care. Additionally, the study has highlighted key correlations that deepen our understanding of neonatal health, particularly the increased vulnerability of extremely preterm infants and those with lower birth weights to severe HIE and IVH. These insights are invaluable for advancing neonatal clinical practices and research, em-phasizing the need for early intervention and comprehensive monitoring in managing at-risk neonates.