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DOCTORAL THESIS

**CORRELATION BETWEEN HISTOCHEMICAL
APPEARANCE OF THE UMBILICAL CORD AND
COMPLICATIONS OF PREMATURITY**

– A B S T R A C T –

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CHAPTER 1: RISK FACTORS ASSOCIATED WITH RETINOPATHY OF PREMATURITY IN VERY AND EXTREMELY PRETERM INFANTS.

CONTEXT

Retinopathy of prematurity (ROP) is a significant cause of visual impairment in children and a principal cause of blindness in preterm infants. ROP is a vascular disease that impacts the developing retina in premature infants. It presents a variety of clinical symptoms, from spontaneous regression to total bilateral detachment of the retina and blindness. The disease progresses through five stages, where Stage 1 and 2 are considered mild and Stage 3 to 5 are considered severe or needing treatment. All stages of ROP have been linked to a decrease in retinal sensitivity, while treatment-requiring ROP also correlates with a reduction in retinal responsiveness. The progression of all stages of ROP and its incidence rates have fluctuated across the globe in the last 10 to 20 years.

The incidence of ROP is roughly 60% in preterm infants with a birth weight less than 1500 g, and this has remained consistent in most studies over time. The progression to severe ROP (Stages 3–5) is reported to be around 15%. The majority of severe ROP cases occur in infants with a birth weight of less than 1251 g. Aside from ROP, a birth weight of less than 1000 g has also been strongly associated with RDS (76% incidence) and a mortality rate of 55% in preterm infants. The incidence of ROP can also be influenced by the gestational age of the premature infants that survive.

Preterm infants are categorized based on their gestational age as moderate, very, and extremely preterm if the gestational age is 32-<37, 28-<32, and <28 weeks respectively. The survival of very and extremely preterm infants is made possible by tracheal intubation techniques used in neonatal intensive care units, however, these must be carefully controlled to manage oxygen intake. Optimal oxygenation to support normal development of preterm infants without affecting postnatal retina development has been researched and established.

Gestational age and birth weight, in addition to oxygen administration, are major risk factors for ROP and form the basis of most ROP screening guides. Research into the physiopathology of ROP has also suggested the involvement of several other factors, including maternal factors such as preeclampsia and chorioamnionitis, prenatal, perinatal, demographic, genetic, and factors related to medical treatment and nutrition.

Understanding the role of these risk factors in the onset and progression of ROP can improve its screening and treatment. A promising approach involves studying the relationships between these risk factors and the different stages of the disease. Although previous studies have extensively researched the association between ROP and various risk factors, fewer studies focus on the link between these risk factors and different stages of the disease. This study examines the correlation between multiple risk factors and the incidence of stages 1 to 3 of ROP in an East European population, contributing to the broader understanding of how ROP and its various stages are influenced by these different risk factors..

RESULTS

This study involved 247 infants with a gestational age (GA) of less than 32 weeks and a birth weight (BW) of 2560 grams or less, screened for Retinopathy of Prematurity (ROP). The incidence of ROP (Stage 1-3) was 66.40% (164 infants), with 15.38% (38 infants), 27.53% (68 infants), and 23.48% (58 infants) for Stages 1, 2, and 3, respectively. Notably, 70.54% of males and 61.86% of females were diagnosed with some stage of ROP.
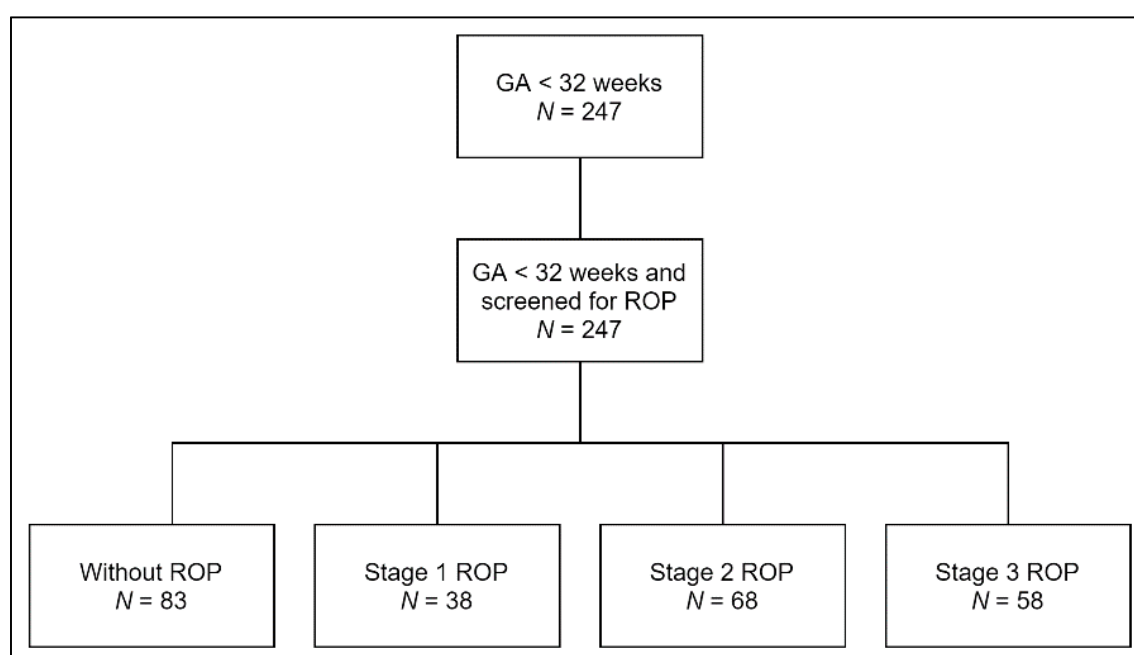

Figure 1 – Distribution of infants in the study.

The study also revealed that the incidence of ROP gradually decreased with increased GA and BW. The rates were 100% for 24-26 weeks, 91.67% for 27 weeks, 84.21% for 28 weeks, 76.92% for 29 weeks, 65.79% for 30 weeks, and 49.56% for 31 weeks. For birth weight groups, the rates were 100.00% for infants weighing less than 750 grams, 92.73% for 750-999 grams, 84.62% for 1000-1250 grams, and 50.00% for those over 1250 grams at birth.

Higher incidence rates of ROP were also found among infants who underwent treatments like ventilation (85.87%), CPAP (78.52%), and surfactant treatment (83.18%), compared to those who did not receive these treatments (54.84%, 51.79%, and 53.57% respectively). Statistical analysis showed that higher GA and BW were associated with decreased odds of ROP (0.530 and 0.997, respectively), whereas undergoing treatments such as artificial ventilation, CPAP, or surfactant treatment increased the odds (5.005, 3.403, and 3.403, respectively).

In the multivariate analysis, birth weight and ventilation treatment emerged as significant risk factors for ROP, with birth weight being negatively correlated and ventilation being positively correlated. Specifically, birth weight was found to be a nearly significant predictor for Stage 1 ROP and a significant predictor for Stages 2 and 3, with higher BW being

associated with lower likelihood of ROP. Ventilation treatment, on the other hand, was a significant predictor for Stage 2 ROP, but didn't hold statistical significance for Stages 1 and 3.
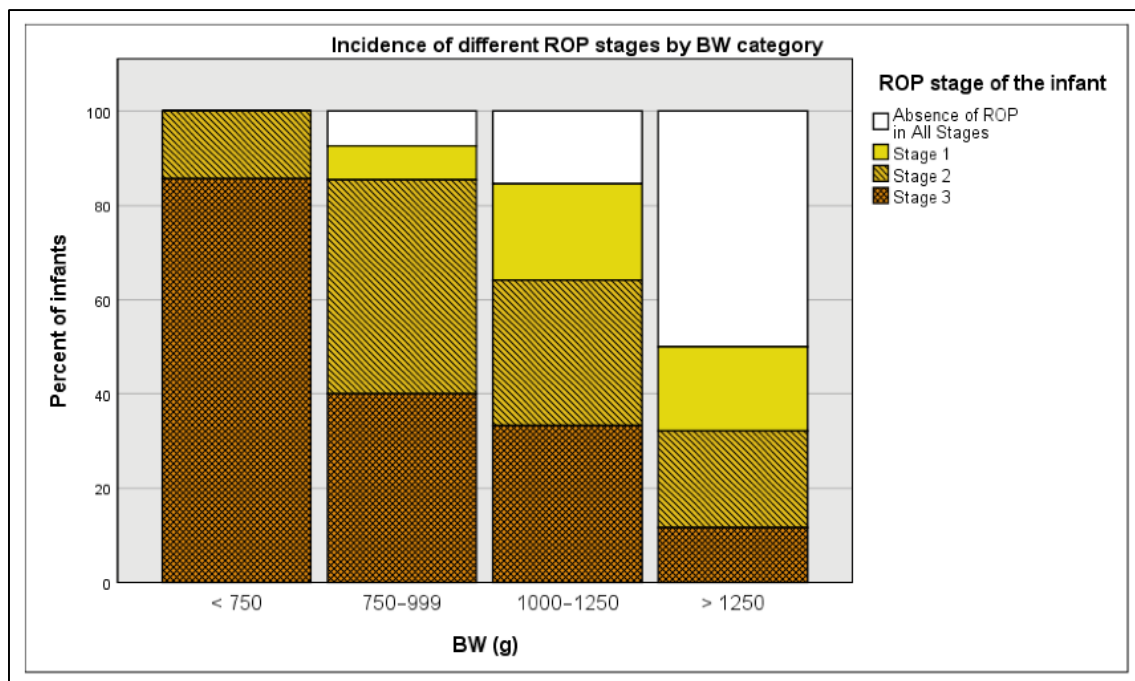

Figure 2 – Percent incidence of various ROP stages stratified by BW groups.

CONCLUSIONS

This study provides confirmation of several well-established risk factors for retinopathy of prematurity (ROP), including gestational age (GA), birth weight (BW), and interventions such as ventilation, continuous positive airway pressure (CPAP), and surfactant administration. In the univariate statistical model, all of these factors demonstrated a significant association with ROP (Stages 1-3). However, when considering multiple factors simultaneously in the multivariate models, only BW and ventilation emerged as significant predictors of ROP (Stages 1-3) and specific stages of the disease, such as Stage 2 and Stage 3 ROP, respectively.

The findings of this study underscore the importance of considering multiple risk factors simultaneously in the analysis of ROP. The multivariate models allow for a more comprehensive evaluation of the independent contributions of various factors while accounting for their interrelationships. By incorporating these factors into predictive models, clinicians can enhance their ability to identify preterm infants at higher risk for ROP and implement targeted interventions accordingly.

CHAPTER 2: THE MOLECULAR AND HISTOPATHOLOGICAL ASSESSMENT OF INFLAMMATORY STATUS IN VERY AND EXTREMELY PREMATURE INFANTS: A PROSPECTIVE STUDY.

CONTEXT

In preterm deliveries, the frequency of histologically diagnosed chorioamnionitis is roughly fifty percent, and its prevalence is negatively correlated to the mother's gestational age. Chorioamnionitis might potentially bring on premature birth since it triggers an inflammatory reaction in the mother. It is also possible for the developing brain to sustain damage from sensitivity to inflammation, and this is especially the case when histologic chorioamnionitis is coupled with fetal inflammatory reaction (FIR). Since these veins are connected with the fetal circulation, any sign of mural inflammatory reaction in the UC vasculature or the chorionic plate blood vessels can imply the presence of FIR. Between fifty and seventy percent of premature placentas that have chorioamnionitis on histological examination also have funisitis. Chorionic plate inflammation affects between 6.15% and about 30% of women. According to some estimates, FIR occurs in anywhere between 25 and 40 percent of all premature newborns. On the other hand, the intensity of the FIR has not been described in a uniform manner.

Research has been conducted on a wide variety of cytokines and associated inflammatory indicators in correlation with premature birth. Some have been investigated to see whether or not they are associated with FIR. The inflammatory markers IL-6 and IL-8 are the ones that have been linked to clinical and histologic chorioamnionitis the most often. Additional indicators of infection include interleukin-1 beta and tumor necrosis factor beta. It has not been documented how sensitive these indicators are in comparison to other immunologic markers when it comes to identifying placental inflammatory alterations. Other possible FIR indicators include enzymes and receptors that participate in the cytokine cascade as chemokines and signaling molecules. The inflammation in the UC and FIR is both strongly connected with fetal complications, such as early onset neonatal sepsis (EONS) and chorioamnionitis due to maternal inflammatory response. As a result, it is conceivable that both the magnitude of the FIR or the frequency of EONS will grow in proportion to the development of an UC inflammatory process. Nevertheless, there have only been a few of investigations that have looked at how the amplitude of the FIR relates to the development of inflammation in the UC. In addition, their findings were conflicting among research findings, which prevented them from concluding that the FIR continuously increases based on the development of inflammatory process in the UC from umbilical phlebitis via engagement of an umbilical artery that can extend further into Wharton Jelly. It is important to mention that most research had limitations in either the categorization of UC inflammatory processes or the studied population.

This study aimed to analyze the correlation among FIR in the context of preterm birth, as assessed by histochemical analysis of the umbilical cord, associated or not with funisitis. The main goal was to determine the level of inflammation of very and extremely premature neonates in correlation with the histological findings of the umbilical cord, while the secondary objective was to analyze the inflammatory markers from the neonates' blood, as determinants for FIR..

RESULTS

The study involved 30 participants, comprised of ten extremely premature infants (EPIs) and twenty very preterm infants (VPIs). As expected, there was a significant difference in average birth weight, with EPIs weighing an average of 871.5 g compared to 1502.0 g for the VPIs. About 70% of the EPI group was born with a weight between 500 and 1000 g, whereas in the VPI group, 55% were born with a weight between 1000 and 1500 g. Of the total participants, there were 16 males and 14 females. Among EPIs, 20% were born at 24 and 25 weeks of gestation, while 85% of VPIs were born at 30 and 31 weeks of gestation.

Inflammatory markers were observed and compared by the level of prematurity. Notably, IL-6 levels at birth were substantially higher among EPIs compared with VPIs, (638.2 vs. 151.1 pg/mL). Conversely, CRP levels at birth were not significantly different between the groups, but after 4 days, CRP increased significantly among VPIs (11.0 mg/dL compared to 7.2 mg/dL in the EPI group). Lactate dehydrogenase levels were more elevated among the EPIs, both at birth and 4 days post birth. Interestingly, the proportions of infants with pathologically increased inflammatory markers did not differ between EPIs and VPIs, regardless of IL-6, CRP, or LDH.

The inflammatory markers at birth and four days post birth between EPIs and VPIs were then compared. The mean LDH levels increased significantly after four days in both the EPI group (from 851.8 UI/L to 962.3 UI/L) and the VPI group (from 468.9 UI/L to 565.9 UI/L). However, the CRP levels increased significantly only among VPIs (from 4.6 mg/dL to 11.0 mg/dL), despite the EPI group being born at an extremely preterm stage.

Histopathology findings from the umbilical cord were compared according to the degree of prematurity. The stage of inflammation did not significantly differ between the EPIs and VPIs. Most newborns were diagnosed with Stage 0 inflammation at the site of the umbilical cord, (40% in the EPI group vs. 55% in the VPI group). There were 4 (40%) infants in the EPI group and 6 (30%) in the VPI group with severe (S3 and S4) inflammation. Regarding histopathological findings, 50% of the EPI infants were identified with Stage 1 FIR, compared to 65% in the VPI infants, with no significant differences observed.
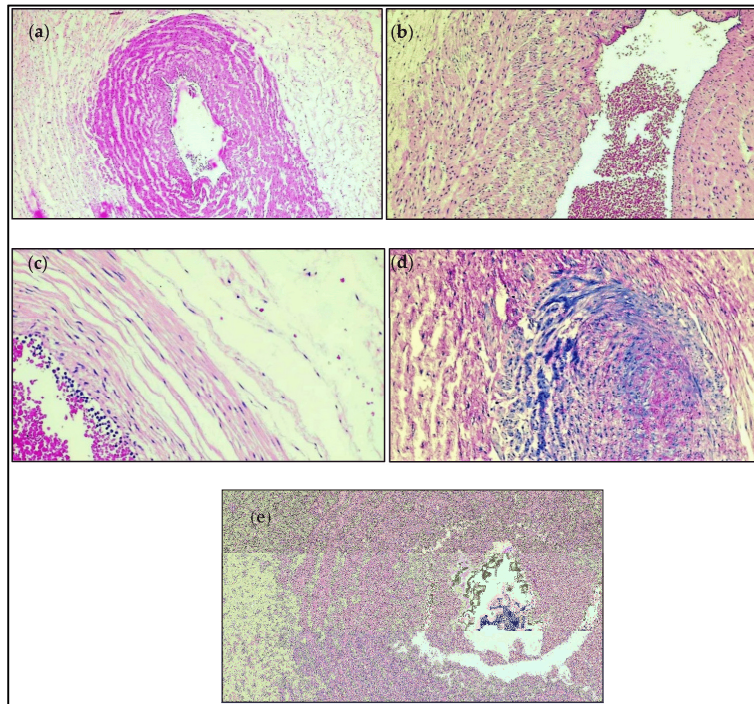
Figure 3 – Histopathological findings: (a)—Stage 0 UC inflammation; (b)—Stage 1 UC inflammation; (c)—Stage 2 UC inflammation; (d)—Stage 3 UC inflammation; (e)—Stage 4 UC inflammation.

Finally, a correlation analysis identified numerous significant associations between the study variables. Gestational age was positively associated with the infants' weight, and negatively correlated with IL-6 and LDH levels. The infants' weight also negatively correlated with IL-6 and LDH levels. The stage of umbilical cord inflammation had a direct correlation with IL-6 and LDH, but not with CRP.

	Weight	Sex	GA	IL-6	CRP	LDH	Stage
Weight	1	0.239	0.418	-0.349	0.157	-0.261	-0.085
Sex	0.239	1	0.042	0.181	-0.054	0.086	-0.038
GA	0.418	0.042	1	-0.340	0.261	-0.259	-0.275
IL-6	-0.349	0.181	-0.340	1	0.215	0.307	0.461
CRP	0.157	-0.054	0.261	0.215	1	0.241	0.197
LDH	-0.261	0.086	-0.259	0.307	0.241	1	0.293
Stage	-0.085	-0.038	-0.275	0.461	0.197	0.293	1

Figure 4 – Correlation analysis.

CONCLUSIONS

The concentration of inflammatory markers and cells present in the cord blood of neonates tends to increase as the gestational age decreases. This relationship, which has been documented in our current investigation, establishes an important premise that inflammatory conditions in neonates can be inferred through the degree of inflammation indicated by these markers in the cord blood.

The use of inflammatory markers as a diagnostic tool is especially advantageous given the implications of these markers in various inflammatory conditions that could be hazardous for neonates, particularly preterm ones. Early detection and understanding of these markers can inform timely intervention and management strategies, potentially mitigating the adverse outcomes associated with such conditions.

A significant advantage of using inflammatory markers in the cord blood as a diagnostic tool lies in its ease of administration. Unlike histopathological analysis of umbilical cord (UC) samples, which can be time-consuming and costly, the examination of inflammatory markers can be conducted rapidly, often within minutes. This speed and efficiency are critical in clinical settings where timely diagnosis can profoundly impact patient management and outcomes.

Moreover, the analysis of these markers offers a comprehensive overview of the extent of funisitis. Funisitis, a condition characterized by inflammation of the umbilical cord, can serve as a valuable indicator of the extent of a fetal inflammatory response. By understanding the degree of funisitis, healthcare professionals can gain insights into potential complications and tailor therapeutic strategies accordingly.

Additionally, the prompt analysis of these markers can guide the clinical decision-making process regarding the management of preterm newborns. The information obtained can aid in determining the appropriateness and timing of administering specific treatments such as corticosteroids and NSAIDs. These medications have been shown to improve survival rates in preterm newborns by promoting lung maturation and reducing inflammation levels that may lead to the fetal inflammatory response.

CHAPTER 3: A PROSPECTIVE ANALYSIS OF THE RETINOPATHY OF PREMATURITY CORRELATED WITH THE INFLAMMATORY STATUS OF THE EXTREMELY PREMATURE AND VERY PREMATURE NEONATES.

CONTEXT

The pathogenesis of ROP is multifactorial, involving a complex interplay of various factors such as oxygen levels, vascular growth factors, and inflammatory mediators. Inflammation has been suggested to play a crucial role in developing ROP, with studies showing elevated levels of pro-inflammatory cytokines in the vitreous humor and serum of

infants with ROP. However, the specific role of inflammation in the pathogenesis of ROP, and its association with the severity of the disease, remains unclear.

Extremely premature and very premature neonates are known to be at a higher risk of developing ROP due to their underdeveloped retinal vasculature and their increased susceptibility to oxidative stress and inflammation. In these infants, the immature immune system and exposure to various stressors, such as infections and mechanical ventilation, can result in a heightened inflammatory response, further contributing to the development and progression of ROP.

Several studies have investigated the association between inflammatory markers and the risk of ROP, focusing on specific cytokines, such as interleukin (IL)-6, IL-8, tumor necrosis factor-alpha (TNF-α), and insulin-like growth factor (IGF). However, the results have been inconsistent, with some studies showing a significant association between elevated cytokine levels and ROP, while others have not found any correlation. Furthermore, most of these studies have been retrospective, with a limited ability to establish a causal relationship between inflammation and ROP.

Given the inconsistencies in the literature and the importance of understanding the role of inflammation in ROP, there is a need for prospective, longitudinal studies that can assess the inflammatory status of extremely premature and very premature neonates and determine its association with the development and progression of ROP. Therefore, the present study aims to conduct a prospective assessment of the development of ROP and its relationship with the inflammatory status of extremely premature and very premature neonates. By longitudinally evaluating the levels of various inflammatory markers, we hope to better understand the role of inflammation in the pathogenesis of ROP and identify potential biomarkers for predicting the risk of ROP in these vulnerable infants.

RESULTS

The study focused on a total of 48 neonates, split into Extremely Premature Infants (EPI) with a gestational age of less than 28 weeks (12 in number) and Very Premature Infants (VPI) with a gestational age between 28 and 32 weeks (36 in number). The two groups were compared in terms of their demographic and clinical characteristics. The average birth weight across all participants was 1291.8 g, with a standard deviation of 405.8. A significant difference was observed in mean birth weight between the EPI and VPI groups (864.5 ± 231.4 g vs. 1392.7 ± 364.2 g, respectively). The EPI group was more likely to fall within the 500–1000 g range (66.7%) compared to the VPIs (19.4%), while all the neonates in the 1500–2000 g range were from the VPI group.

Regarding C-reactive protein (CRP) levels at birth, no significant difference was found between the groups. However, CRP levels at three days were notably higher in the VPI group (11.0 ± 1.3 mg/dL) compared to the EPI group (7.2 ± 3.2 mg/dL). Lactate Dehydrogenase (LDH) levels, both at birth and at three days, were significantly higher in the EPI group

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compared to the VPI group, with mean LDH levels at birth being 851.8 ± 72.2 UI/L in the EPI group and 468.9 ± 108.2 UI/L in the VPI group.

Leukocyte counts at birth and three days were higher in the EPI group compared to the VPI group. The percentage of Polymorpho-nuclear leukocytes (PMNs) at birth was higher in the VPI group (47.2 ± 20.9%) compared to the EPI group (33.4 ± 16.5%). Regarding the prevalence of abnormal laboratory findings, all the EPIs (100%) had pathological levels of Interleukin 6 (IL-6), compared to 77.8% of the VPIs. C-reactive protein (CRP) levels were similar in both groups, with 83.3% of EPIs and 86.1% of VPIs presenting abnormal levels.

Pathologically high levels of Lactate Dehydrogenase (LDH) were observed in both groups, with 91.7% of EPIs and 83.3% of VPIs showing abnormal levels. When considering leukocyte counts, 66.7% of EPIs and 52.8% of VPIs had abnormal counts. Lastly, the polymorphonuclear leukocytes (PMN%) proportion was pathologically high in 75.0% of EPIs and 55.6% in VPIs.
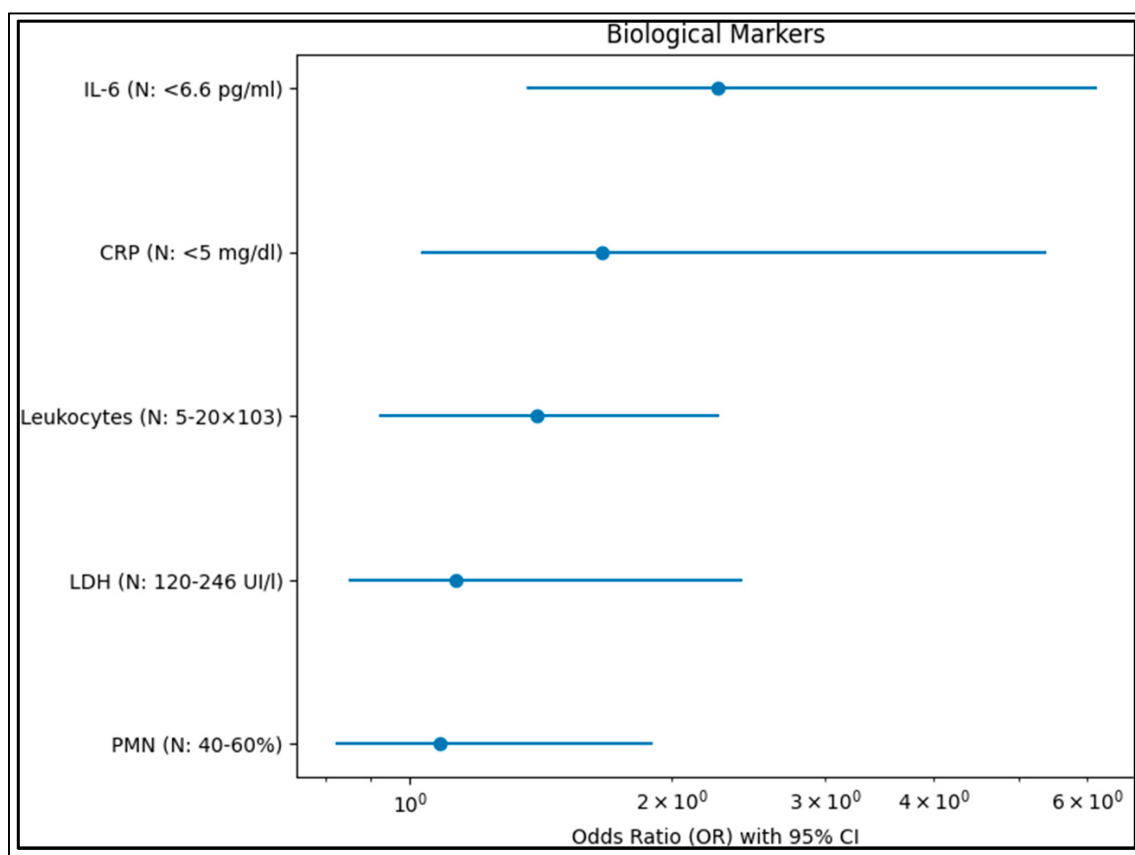

Figure 5 – Biological markers identified as risk factors for ROP stage 2 or above.

CONCLUSIONS

The findings of this study underline the critical role inflammation plays in the development and progression of Retinopathy of Prematurity (ROP) in extremely premature and very premature neonates. Our data confirm the initial hypotheses that higher levels of

inflammatory markers are associated with an increased risk of ROP development and progression and that neonates with ROP exhibit a distinct inflammatory profile compared to those without. The significant disparities in birth weight, IL-6 levels, CRP levels, and LDH levels between extremely and very premature infants underscore the effect of gestational age and birth weight on inflammatory status. Furthermore, our findings revealed that the duration of oxygen supplementation, mechanical ventilation, prolonged CPAP use, gestational age of fewer than 28 weeks, and umbilical cord inflammation at or above stage 3 were significant risk factors for the development of ROP stage 2 or above.

Elevated levels of CRP and IL-6 were also significantly associated with an increased risk of developing ROP stage 2 or above, highlighting their potential as biomarkers for ROP risk prediction. Overall, this study underscores the crucial need for early and consistent monitoring of inflammatory markers in premature neonates and points towards the potential of inflammation-targeted therapeutic strategies in mitigating the risk and severity of ROP in this vulnerable population. Future research should focus on validating these findings in larger, multi-center cohorts and exploring the mechanistic pathways linking inflammation and ROP to refine our understanding further and enable the development of targeted interventions.