„VICTOR BABEȘ” UNIVERSITY OF MEDICINE AND PHARMACY FROM TIMISOARA
FACULTY OF MEDICINE
DEPARTMENT OF OBSTETRICS AND GYNECOLOGY

PANTEA MANUELA-MARINA

PhD THESIS

BIOMARKERS USED IN DIFFERENTIATING SEPSIS FROM SYSTEMIC INFLAMMATORY RESPONSE SYNDROME IN NEONATES

- ABSTRACT -

Scientific leader
PROF. UNIV. DR. IACOB DANIELA

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Neonates, a critical patient group, often face the challenge of adapting to extrauterine life after intrauterine events. These events can have short-term and long-term clinical consequences, shaping subsequent management decisions. Inflammation, a significant factor in preterm and term birth, can lead to complications and mortality. Fetal Inflammatory Response Syndrome (FIRS), a pathological condition caused by fetal exposure to infection or injury, can result in multi-organ damage and neonatal mortality. FIRS is also associated with delivery at term, making it uncharacteristic for prematurity. FIRS can be further described as associated with infection or considered sterile, like the systemic inflammatory response (SIRS). The immaturity of the innate immune system makes it difficult to distinguish between sepsis and SIRS, as does therapeutic management.

Neonatal sepsis represents a significant global healthcare burden, with approximately 1.4 million neonatal deaths annually. Preterm infants are particularly susceptible to infection and have a higher risk of complications and long-term mortality than term infants. The diagnosis and prognosis of neonatal sepsis remain challenging due to the lack of specificity of clinical signs and symptoms and the suboptimal predictive ability of routine laboratory tests.

This study holds great promise as it aims to differentiate infectious from non-infectious pathologies by determining serum markers in dynamics. The correlation of identified serum markers with the two pathologies is crucial for further developing targeted therapeutic management and reducing antibiotic use without a positive diagnosis, offering a hopeful future for neonatal care.

This doctoral thesis is divided into two sections. The general part is centered on exploring the neonatal period, including short-term and long-term complications, and the systemic inflammatory response syndrome with its guidelines. The general part relies on an extensive review of pertinent literature. The special part, the most extensive and detailed section of the thesis, is devoted to presenting and analyzing three studies, each approaching the subject from a specific perspective. The special part follows up on identifying potential biomarkers for diagnosing SIRS in newborns, emphasizing their predictive value.

The first chapter of the special part represents the study conducted on premature newborns. Given that prematurity is the leading cause of neonatal morbidity and mortality, it is of particular importance to diagnose complications early and initiate targeted treatment. Preterm infants developing SIRS is a matter of concern, as it can rapidly escalate to critical complications like sepsis, multi-organ dysfunction syndrome, and even death. Furthermore, clinical appearance and laboratory findings are usually nonspecific and late-appearing in this population, leading to delays in diagnosis and treatment. Various biomarkers have been studied, considering an earlier diagnosis, including acute-phase proteins, cytokine, and hematological ratios. This study aimed to evaluate the predictive and diagnostic capability of laboratory markers like NLR, derived NLR, PLR, and NLPR in diagnosing SIRS in premature newborns. Methods: Premature newborns with and without SIRS were evaluated in a prospective design for one year based on inclusion and exclusion criteria. The classification of the newborns into two groups – no SIRS and SIRS – relied on the current guidelines. The collection of blood samples was deliberately organized at two specific periods after birth. The first

collection was conducted within 24 hours after birth, and the second at 72 hours after birth. The final study included 136 preterm newborns, divided into 53 preterm newborns who developed SIRS and 83 preterm newborns who did not.

Results: At 24 hours, the NLR and dNLR values were significantly higher in the SIRS group, highlighted by p-values of 0.030 and <0.001, respectively, while laboratory parameters including LDH, CK, AST, and ALT showed no significant difference between the two groups. NLR's cutoff value was 8.69, yielding sensitivity and specificity rates of 52.77% and 83.47% (p=0.0429), respectively. The dNLR showed a cutoff of 5.61, with corresponding rates of 63.27% and 84.15% (p=0.0011), PLR had a cutoff of 408.75, with rates of 51.89% and 80.22% (p=0.1026), and NLPR displayed a cutoff of 0.24, with rates of 75.85% and 86.70% (p=0.0002). At 72 hours, WBC count and neutrophil values were higher in the SIRS group than in no SIRS, establishing a p-value of 0.001. Notable sensitivity and specificity improvements were observed, particularly with the NLR having a cutoff of 4.4, showing specificity of 91.57% (p=0.004), and the NLPR having a cutoff of 0.17, showing a sensitivity of 77.74% and specificity of 95.18% (p<0.0001). The regression analysis demonstrated that ratios above the best cutoff value had different hazard ratios. NLR above the cutoff indicated a 33% increase in SIRS risk (HR 1.33). The dNLR was associated with a twofold increase in risk (HR 2.04). NLPR demonstrated a significant, over threefold increase in SIRS risk (HR 3.56), underscoring its substantial predictive and diagnostic value for SIRS development. The first study concludes that integrating these findings into clinical practice could enhance neonatal care by facilitating the early

identification and management of SIRS, potentially improving outcomes for this vulnerable population.

The second chapter of the special part is designated to the research conducted on full-term newborns. The neonatal period is critical for newborns, establishing the foundation for long-term health and development. The immune system of neonates is a dynamic and evolving structure characterized by enhanced sensitivity to a broad spectrum of conditions. Systemic Inflammatory Response Syndrome in newborns can be caused by various factors, including bacterial, viral, or fungal infections, as well as non-infectious causes such as trauma or ischemia. SIRS and consequent sepsis are less frequent in full-term neonates compared to preterm infants, but they still pose a substantial risk. The present study hypothesizes that increased levels of hematologic ratios and changes in liver function tests serve as significant indicators of SIRS in term newborns. Hence, the objectives are twofold: first, to confirm the predictive value of Neutrophil to Lymphocyte Ratio (NLR), Derived Neutrophil to Lymphocyte Ratio (dNLR), Platelet to Lymphocyte Ratio (PLR), Neutrophil, Lymphocyte, and Platelet Ratio (NLPR), AST to Platelet Ratio Index (APRI) and Systemic Inflammation Index (SII) in identifying the risk for SIRS development in full-term newborns. Secondly, to evaluate the link between liver function parameters and the severity of SIRS in this particular population. This observational cohort study compared full-term newborns diagnosed with SIRS with newborns without SIRS based on inclusion and exclusion criteria. In the study's context, the diagnosis of SIRS was considered based on existing guidelines. The study focused on newborns delivered at a gestational age of 37 weeks or above who were admitted to the neonatal intensive care unit. Blood

samples were collected at two different periods after birth, 24 hours and 72 hours, to observe the levels of inflammatory and liver function markers and their dynamic changes. The study included 229 newborns, 81 with SIRS and 148 without SIRS. Statistically significant differences with p-values under 0.0001 were observed in the first 24 hours regarding the following laboratory parameters: lactate levels, WBC, neutrophils, lymphocytes, platelets, C reactive protein, LDH, AST, ALT, NLR, dNLR, PLR, NLPR, APRI, SII. At the second time point, 72 hours after birth, differences in laboratory values between neonates with and without SIRS persisted, showing statistical significance with p-values under 0.0001. The inflammation scores were compared between the control and SIRS groups at 24 and 72 hours of life. Moreover, the comparison extended to include the calculation of the ratios distinctly for viral and bacterial infection from the SIRS group. The ratios (NLR, dNLR, PLR, NLPR, APRI, SII) exhibited a progressive increase from no SIRS to bacterial infection, suggesting a more robust systemic inflammatory response linked to bacterial infections. On the first day of life, the highest sensitivity showed SII at 72.13% ($p=0.001$), while APRI demonstrated the highest specificity at 82.45% ($p=0.037$). The NLPR demonstrated substantial diagnostic value, with a sensitivity of 78.36% and specificity of 83.52% at 72 hours ($p<0.0001$). Regression analysis highlighted that NLPR and SII were strongly predictive of SIRS, with NLPR showing over three times higher SIRS risk (HR 3.29, $p<0.0001$) and SII indicating nearly 3.5 times the risk (HR 3.47, $p<0.0001$). Inflammatory markers like NLR, PLR, NLPR, and SII, alongside liver function tests, are significant predictors of SIRS in full-term newborns. These findings support the integration of these markers into routine neonatal care, allowing for early identification and potentially

improved management of newborns at risk for SIRS, thereby enhancing clinical outcomes.

The third chapter of the thesis compiles the results obtained from assessing the predictive role of maternal laboratory values sampled during pregnancy and the risk of their newborns developing SIRS in the neonatal period. Maternal laboratory findings and scores can be valuable predictors of neonatal sepsis and systemic inflammatory response syndrome. Elevated maternal C-reactive protein levels significantly increase the risk of early-onset neonatal sepsis, and maternal complete blood count is an essential parameter for predicting early-onset neonatal sepsis. Maternal hematological scores, such as Neutrophil-to-Lymphocyte Ratio (NLR) and Platelet-to-Lymphocyte Ratio (PLR), emerged as promising parameters as predictors for preterm delivery and neonatal outcome. This study aimed to identify maternal laboratory parameters predictive of SIRS in newborns, focusing on establishing diagnostic cutoffs and evaluating the predictive power of these biomarkers. This prospective cohort study was conducted from one year, January 2023 to January 2024. It included 207 mother-newborn pairs, divided into groups based on the neonatal development of SIRS (66 cases) or its absence (141 controls). The study focused on pregnant women who had investigated pregnancies so that maternal investigations were followed. The inclusion in this study was not based on gestational age; therefore, delivering at any gestational age was included. The maternal blood samples were collected at two specific time points: during the third trimester and immediately at delivery. These dual collections aimed to observe the dynamic variations in the maternal biochemical environment that could affect neonatal outcomes, specifically the

development of SIRS. Key maternal parameters measured included inflammatory markers and liver enzymes, analyzed using standard biochemical methods. At the end of the third trimester, regarding inflammatory scores, all metrics exhibited a notable increase in the SIRS group. The neutrophil-to-lymphocyte ratio (NLR), derived neutrophil-to-lymphocyte ratio (dNLR), platelet-to-lymphocyte ratio (PLR), neutrophil-to-lymphocyte-to-platelet ratio (NLPR), AST-to-platelet ratio index (APRI), ALT-to-lymphocyte ratio index (ALRI), and the systemic immune-inflammation index (SII) were all significantly increased with p-values below 0.001. This trend persisted 24 hours after delivery, showing the same statistical significance. Receiver operating characteristic (ROC) analysis was applied to establish optimal cutoff values, and multivariate logistic regression was used to determine hazard ratios (HR) for SIRS prediction, with adjustments for potential confounders. The study identified significant ROC/AUC values for several biomarkers. The neutrophil-to-lymphocyte ratio (NLR) demonstrated an AUC of 0.926, with a cutoff value of 3.64, achieving 81.8% sensitivity and 90.9% specificity ($p < 0.001$). The Systemic Immune-Inflammation Index (SII) showed an AUC of 0.819 and a cutoff of 769.12, with 75.8% sensitivity and 81.8% specificity ($p < 0.001$). Multivariate regression analysis highlighted that neonates with maternal SII values above this cutoff were three times more likely to develop SIRS (HR 3.09, 95% CI 2.21–4.17, $p < 0.0001$). Other notable biomarkers included dNLR and ALRI, with respective HRs of 1.88 ($p = 0.018$) and 1.75 ($p = 0.032$). These findings confirm the significant predictive value of specific maternal inflammatory markers for neonatal SIRS. These findings support the utility of these biomarkers in prenatal screening to identify neonates at increased risk of SIRS, potentially guiding preemptive clinical interventions.

The immune system of newborns is a constantly changing and developing structure highly responsive to various situations. The increased susceptibility is mainly ascribed to the continuous maturation of the immune system as it transitions from the intrauterine environment to the microorganism-filled exterior environment. The innate immune system is essential in establishing a dependable defense in neonates. Interestingly, despite its relative lack of specificity, the innate immune system can effectively distinguish self from non-self and provide defense to a wide range of pathogens. Neonates, who have an underdeveloped adaptive immune response compared to adults, greatly benefit from this.

Nevertheless, the underdeveloped immune system characterizes both full-term and preterm neonates. Therefore, the aim of this study aligned with the initial interest in facilitating diagnostic methods by orienting them towards rapidity, specificity, sensitivity, and improving patient outcomes. The validation of inflammatory ratios in adult patients has prompted research in the pediatric and neonatal populations, with hematologic inflammatory scores showing promise in predicting SIRS. These scores have several advantages, including their derivation from a standard laboratory test (complete blood cell count) and noninvasive nature. Future research is desirable in hopes of finding a diagnostic protocol containing clinical characteristics and ideal biomarkers. An optimal one with increased sensitivity, specificity, and positive and negative predictive values has yet to be determined. Thus, the results of these studies, which include hematologic inflammatory scores, add value to the early determination of SIRS in neonates. As predictive tools for determining SIRS, hematologic ratios are gaining strength, advocating their implementation into standard neonatal care assessment.

The research conducted in this doctoral study has provided valuable insights into neonatal systemic inflammatory response syndrome and the prognostic value of various laboratory findings. The results strongly support the incorporation of these biomarkers into standard neonatal care, which can improve outcomes through timely and accurate medical interventions. However, further research involving larger cohorts and exploration of the clinical applicability of these biomarkers is necessary. Future perspectives should focus on investigating these hematologic ratios in a larger group of preterm newborns, considering their gestational age and birth weight. As newborns are also affected by SIRS and sepsis, future research should also encompass this population. Future studies must identify and develop diagnostic tools that are sensitive and specific for SIRS and sepsis in the neonatal population, with the ultimate aim of enhancing neonatal outcomes.