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BIOLOGICAL CHANGES ASSOCIATED WITH PREMATURE BIRTH AND TERM BIRTH

ABSTRACT

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SUMMARY

Preterm birth is a major global health issue that affects more than 15 million newborns each year. In addition, approximately 1 million children die each year as a result of preterm birth complications. Besides being the leading cause of mortality in infants under 28 days of age, it also contributes to increased prevalence of severe neurodevelopmental disorders, or many have less severe disabilities and inadequate child development. Given the enormous personal, economic, and health consequences of preterm birth, predictive tests are critical. These are important for identifying women who are at an increased likelihood of experiencing a preterm birth in order to provide prophylactic interventions and guide prenatal management decisions.

Preterm birth is a complex syndrome that can be caused on by a variety of facts. Stress, maternal infections, placental ischaemia or vascular lesions, and uterine overdistension are all common causes of preterm birth. These pathways differ in terms of beginning and mediating factors, but they all result in early uterine contractions and preterm birth. Although the exact mechanisms causing spontaneous premature onset of birth remain unclear, it is known that receptors, chemokines, and inflammatory cytokines are involved

The pregnancy period comes with a series of physiological adaptations that are well-known to be associated with different immune processes and elevated inflammatory status of the pregnant woman. Since inflammation is believed to have a significant role in the initiation of labor in both preterm and term births, prior studies have focused on the variation in inflammatory markers and biological blood parameters to determine the link between serum markers of the pregnant woman and the risk of preterm birth. It has been found that the number of macrophages increases in response to both term and preterm births, while neutrophils are more prevalent in the decidua of individuals with preterm births. Therefore, it is plausible to postulate that an

abnormally increased inflammatory status can trigger the moment of birth before the normal 37 weeks of gestation.

Many of the biological changes, especially those related to inflammation, can be assessed by complete blood count. However, it is known that besides premature birth, normal pregnancy also involves biological changes in the maternal organism, including inflammatory changes. Therefore an assessment of the differences between normal and pathological changes revealed in laboratory blood counts is essential to determine the predictive factors.

In this thesis we analyzed different inflammatory markers, detected from routine laboratory tests, in the prediction of preterm birth. The topic is important and topical, as it has implications for improving the health outcomes of preterm infants and term-born neonates. The relevance of this topic to the international, national, and regional concerns of the research team lies in the high incidence of preterm birth.

The studies covered in this thesis were conducted to investigate the biological changes induced by preterm birth, in comparison with term birth, which can be revealed by laboratory analysis.

Thus, data from these studies may allow the development of improved approaches to assess the risk of preterm birth, leading to appropriate interventions to prevent it. In addition, risk assessment of preterm birth can be clinically implemented resulting in decreased neonatal morbidity through antenatal interventions.

In addition, the research in this thesis aims to further investigate the impact of SARS-CoV-2 infection on pregnant women, with a particular focus on understanding the clinical and biological characteristics that contribute to preterm birth.

The scientific objectives of this doctoral research are to identify and characterize the biological changes associated with preterm birth and term birth, explore the potential impact of these changes on health during

pregnancy and neonatal outcomes, and evaluate the effectiveness of interventions aimed at improving the health outcomes of preterm infants and term-born neonates.

The thesis will be divided into two main sections.

The general part presents the current state of knowledge. This section also provide an overview of the biological changes associated with preterm birth and term birth, including changes in the respiratory, cardiovascular, and immune systems.

The special part will explore the potential impact of these changes on pregnancy outcomes. This section includes three studies whose results have been published in Clarivate Web of Science indexed journals.

Study 1, covered in Chapter 5 of the thesis, aimed to explore the clinical and biological characteristics of pregnant women with SARS-CoV-2 infection during pregnancy in relation to preterm birth. Concerning the link between infections and preterm birth, given the recent pandemic, I also focused my interest in this thesis on SARS-CoV2 infection during pregnancy.

While existing literature has provided some insights into the potential risks of prematurity and low birth weight associated with COVID-19 infection during pregnancy, there is still limited information on the pregnancy outcomes based on the trimester of infection, as well as the clinical and biological characteristics of the pregnant patients that may contribute to premature birth.

The study was performed by following a retrospective observational design that focused on pregnant women who contracted SARS-CoV-2 during pregnancy and were admitted or evaluated at the Obstetrics and Gynecology Clinic of the Timisoara Municipal Emergency Hospital between 1 March 2020, and 31 December 2021.

During the selection process, a total of 428 pregnant women were included in the final analysis. After the selection process, we identified 61 eligible pregnant women with a history of SARS-CoV-2 infection during the last pregnancy that resulted in preterm birth. From this starting point, a matched

group on a 2:1 ratio was selected among pregnant women positive for COVID-19 during their pregnancy who gave birth at full term. A second control group consisting of women who gave birth prematurely without COVID-19 infection was matched 4:1 to the study group.

The patient's medical history and current pregnancy outcomes, identified that the pregnant women with COVID-19 who gave birth prematurely had similar trends with the control group of patients with preterm births without COVID-19 infection. Thus, it was observed that gestational hypertension was a complication more frequently affecting the two prematurity groups (9.8% vs. 9.5% vs. 2.4% in the COVID-19 no prematurity group, p-value = 0.039). Among current pregnancy complications, anemia was statistically significantly more prevalent among the two prematurity groups compared to the control group with full-term births. There was no significant difference between the COVID-19 prematurity group and the control group of no COVID-19 mothers who gave birth prematurely (24.6% vs. 24.3%, p-value = 0.903). Another important finding was that patients who gave birth prematurely had significantly more SARS-CoV-2 infections during the late stages of pregnancy compared with the control group (47.5% of patients with COVID-19 in the d-trimester, compared with 32.3% in the control group with full-term births and COVID-19, p-value = 0.005). Moreover, it was observed that the same patients in the COVID-19 prematurity group were significantly more likely to have a symptomatic infection (76.4% vs. 47.6%, p-value = 0.021).

The white blood cell count was significantly more elevated in the COVID-19 prematurity group, likely due to the late pregnancy infection with SARS-CoV-2 (26.2% elevated samples, vs. 11.3% in the COVID-19 no prematurity group, respectively 15.6% in the no COVID-19 prematurity group, p-value = 0.042). Other significant findings were the presence of anemia more frequently in the COVID-19 prematurity group in 21.3% of patients, compared to the no COVID-19 prematurity group (11.9%, p-value = 0.037). Lastly, the CRP levels were more elevated among pregnant women with SARS-CoV-2

infection who gave birth prematurely, compared with the control group of premature births without a history of COVID-19 (16.4% vs. 7.4%, p-value = 0.024).

A multiple linear regression model was constructed to observe the predictors for premature birth in COVID-19 pregnant women since SARS-CoV-2 infection alone did not show statistical significance in determining a premature birth (β = 1.09, CI = 0.94–1.15, p-value = 0.067). The significant predictors from clinical findings were smoking, gestational hypertension, PROM, UTIs, as well as the SARS-CoV-2 infection during the third trimester of pregnancy (β = 1.55, CI = 1.38–2.93, p-value = 0.014), and a having a symptomatic infection (β = 1.23, CI = 1.09–1.38–2.21, p-value = 0.036). In addition to the significant clinical findings, some biological parameters were found to have a significant influence on the onset of premature birth in association with the SARS-CoV-2 infection. Anemia was the strongest predictor in association with COVID-19 (β = 3.65, CI = 1.46–5.39, p-value < 0.001), followed by elevated CRP (β = 2.11, CI = 1.20–3.06, p-value < 0.001), and respectively IL-6 (β = 1.92, CI = 1.20–2.47, p-value = 0.001).

In conclusion, our findings support the idea that SARS-CoV-2-positive pregnant women do not expose the fetus to an additional risk of intrauterine growth restriction or significant complications. SARS-CoV-2 infection during the third trimester, it is recommended that these patients be admitted to hospital for observation of clinical course and biological parameters such as anaemia and increased inflammatory markers, with additional effect on pregnancy outcome.

Study 2, covered in Chapter 6, aimed to investigate the predictive role of maternal biomarkers and inflammatory scores, including neutrophil/lymphocyte ratio (NLR), platelet/lymphocyte ratio (PLR), monocyte/lymphocyte ratio (MLR), systemic immune-inflammation index (SII) and systemic inflammatory response index (SIRI), in assessing the risk of preterm birth. The research was conducted as an observational study at the

Department of Obstetrics and Gynecology from the Victor Babes University of Medicine and Pharmacy in Timisoara, Romania.

The study comprised 486 patients analyzed for changes in biological parameters during the pregnancy period in order to determine the predictive role of various inflammatory scores computed from the basic serum biomarkers. Patients were split into two equally matched study groups, a reference group of 243 pregnant women who gave birth prematurely and 243 pregnant women who gave birth at full term.

. A total of 209 patients had their blood samples taken during the second trimester of pregnancy, while the other 277 were measured during the third trimester, with no significant changes between the two study groups. Among biological markers, it was observed that the white blood cell count, lymphocyte count, monocyte count, and the number of platelets had significantly different average values. Additionally, anemia was significantly more common among patients with premature birth, with a hemoglobin level of 11.72 g/dL, compared to 12.99 g/dL (p-value < 0.001). Regarding the inflammatory scores calculated for each study group, it was observed that NLR (13.75 vs. 9.06, p-value < 0.001), dNLR (6.92 vs. 5.11, p-value < 0.001), PLR (286.2 vs. 237.0, p-value = 0.007), and MLR (0.86 vs. 0.79, p-value = 0.005) scores were significantly higher among those who had a preterm birth.

ROC analysis and calculated areas under the curve were used to determine the predictive role of calculated inflammatory markers in preterm birth. It was observed that the AUC values of NLR, dNLR, PLR, and MLR were higher than 0.600, respectively NLR had the highest value among the tested scores (AUC = 0.694, p-value = 0.009), with the highest sensitivity in this study (71%). The highest specificity was achieved by dNLR, with 70%, and an AUC value of 0.655 (p-value = 0.022). PLR had the second-highest AUC value (0.682) and the best score in terms of sensitivity (70%) and specificity (69%) (p-value = 0.015). Lastly, MLR had the lowest significant AUC score (0.607) and lowest sensitivity/specificity values among the statistically significant

scores (p-value = 0.048). SII and SIRI scores had computed AUC values below 0.600 without statistical significance. The univariate Cox regression analysis calculated a hazard ratio for premature pregnancy of 3.61 (p-value < 0.001) for an NLR score over 9.0 (log-rank p-value = 0.046). The risk was 3.13 times higher when a dNLR score surpassed the cut-offcut-off value of 9.8 (log-rank p-value = 0.020). The PLR risk was the highest among the calculated scores, with an HR of 4.07 (p-value < 0.001), over the threshold of 250 (log-rank p-value = 0.003). Lastly, an MLR score higher than 0.70 posed a 1.96 times higher risk for premature pregnancy (log-rank p-value = 0.039). The SII and SIRI scores were eliminated from the probability analysis since they did not show significant results.

In conclusion, the inflammatory scores NLR, dNLR, PLR, and MLR measured throughout the second and third trimesters of pregnancy exhibited a high predictive value for preterm delivery. Future clinical research should study techniques to diminish the impacts associated with high levels of these indices in order to enhance therapies and management in order to lessen the burden of preterm.

Study 3, covered in Chapter 7 of this thesis, aimed to assess the usefulness of haemoglobin, haemoglobin-albumin-lymphocyte-platelet score (HALP), and coagulation parameters as predictors of preterm birth.

This study was designed as a retrospective cohort at the Obstetrics and Gynecology Clinic of the Municipal Emergency Hospital in Timisoara, Romania, in affiliation with the University of Medicine and Pharmacy "Victor Babes" in Timisoara, Romania.

At the end of the patient selection process, a total of 322 eligible pregnant women were included in the analysis with a complete background profile and laboratory profile.

It was observed that there was a statistically significant difference between the mean values of lymphocytes in the prematurity and “no prematurity” groups since women who gave birth prematurely had a lower

lymphocyte count than the control group (0.78 × 109/L vs. 1.06 × 109/L, p-value < 0.001). Additionally, hemoglobin levels were significantly decreased among women from the prematurity group (113 g/L vs. 139 g/L, p-value < 0.001). The coagulation parameters PT and aPTT were also decreased in the prematurity group (12.9 s vs. 13.3 s, p-value = 0.041) and 26.7 s vs. 27.9 s (p-value < 0.001), respectively. The average albumin levels were 31.4 g/L among women who gave birth prematurely, compared with 36.6 g/L among those who gave birth at term (p-value < 0.001). Fibrinogen and d-dimers, on the other hand, were significantly more elevated in the prematurity group (229 ng/mL vs. 216 ng/mL, p-value = 0.009) and 5.13 g/L vs. 3.10 g/L in the “no prematurity” group (p-value < 0.001), respectively. The mean HALP score value among women who gave birth prematurely was 12.82, compared to 23.96 in the control group (p-value < 0.001). The FAR score was above the threshold in the prematurity group (0.16 vs. 0.08, p-value < 0.001).

The computed area under the curve (AUC) was above 0.600 in all six studied parameters, although none of them was higher than 0.700. Therefore, it can be considered that they have a poor discrimination, even though they have statistical significance. The highest value was observed to be represented by the HALP score with AUC = 0.680 and the highest sensitivity as well (75%, p-value = 0.001). The highest specificity was achieved by the prothrombin time (69%), and the HALP score was also 69%. The FAR score had an AUC of 0.646, with a sensitivity of 68% and specificity of 64% (p-value = 0.020). All other variables were significant estimates for the risk of preterm birth, although with lower accuracy.

Pregnant women with hemoglobin levels below 120 g/L (12.0 g/dL) had a 3.28 higher likelihood of giving birth prematurely (p-value < 0.001). A prothrombin time below 12.5 s determined a 2.11 times higher risk of preterm birth (p-value = 0.038). Similarly, the aPTT below 25 s was linked with 3.24 higher odds of giving birth prematurely. However, the strongest predictors were the D-dimers above 250 ng/mL (OR = 4.26), the FAR score below 0.1, with an

odds ratio of 5.30, and the HALP score with a 6.09 OR for a cut-off value above 24 (p-value < 0.001).

In conclusion, hemoglobin levels, the association of hemoglobin, albumin, lymphocyte, and platelets' (HALP) score, and coagulation parameters such as the prothrombin time (PT), activated partial thromboplastin clotting time (aPTT), D-dimers, and fibrinogen to albumin ratio (FAR) during the third trimester of pregnancy, proved to be significant determinants for the risk of preterm birth. However, due to financial constraints, it would be more appropriate to assess these predictors only in pregnancies that are at risk. Nevertheless, further external validation is required to confirm the accuracy of these predictors for premature delivery, since they all showed a poor level of discrimination.

To summarise, in the studies presented in this thesis it is shown that preterm birth can be predicted using biomarkers derived from the blood count. The great advantage of these markers is that they can be used even in many hospitals or small clinics without the possibility of using advanced or expensive tests. Thus, I strongly support the direction in which the detection of the risk of preterm birth in Romania should be going is the use of NLR, PLR or HALP biomarkers.

These markers have been shown to be important predictors in many other pathologies. Their usefulness in predicting preterm birth has also been demonstrated. However, a validity on the Romanian population has not been demonstrated until the publication of the studies in this thesis, which demonstrates the originality of this research.

Finally, I support the validation of these studies in other clinics, as these scores have been successfully used in our clinic by several physicians. In addition, I advocate the consideration of these investigations in national government programs to reduce premature birth and therefore neonatal mortality.