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# **PhD THESIS**

**THE PREDICTIVE VALUE OF HEMATOLOGICAL  
BIOMARKERS IN IMPROVING DIAGNOSTIC  
EFFICIENCY IN PEDIATRIC CHRONIC DISEASES**

## **A B S T R A C T**

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# ABSTRACT

## INTRODUCTION

In recent decades, the incidence of chronic diseases among children has risen significantly, with estimates reaching as high as 30%. This escalation has been accompanied by a corresponding increase in the global burden of disability. Emerging evidence suggests that inflammation is a common pathogenic mechanism underlying numerous chronic health conditions, including obesity, diabetes, metabolic syndrome (MetS), cardiovascular disease, cancer, asthma, inflammatory bowel disease, and autoimmune diseases.

Inflammatory biomarkers include both canonical markers like C-reactive protein (CRP), fibrinogen, and serum amyloid A, and more complex markers such as cytokines (e.g., Interleukin-1, Interleukin-6, tumor necrosis factor- $\alpha$ ) and oxidative stress indicators like reactive oxygen species. The inflammatory proteins released during inflammation also influence cellular populations in the hematopoietic system. Consequently, recent studies have focused on inflammation-induced changes in immune cell characteristics, using indices derived from different white blood cell (WBC) subtypes in complete blood counts (CBC). These indices, including the neutrophil-lymphocyte ratio (NLR) and the Systemic Immune-Inflammation Index (SII), have proven their value in investigating chronic inflammatory diseases, especially in adults.

Pediatric obesity and juvenile idiopathic arthritis (JIA) are two examples of chronic diseases where inflammation plays a significant role. Despite progress in medical research, there is still a need for reliable biomarkers that can accurately indicate the inflammatory status and disease activity in pediatric patients with chronic conditions. Exploring CBC-derived indices as potential inflammatory biomarkers addresses this need and offers promise for improving clinical management and outcomes. Therefore, the present thesis focuses on the biological investigations of children with these two chronic diseases, particularly examining CBC parameters and indices and their correlation with commonly used disease biomarkers.

Given the involvement of platelets in endothelial dysfunction, which characterizes obesity associated conditions like elevated blood pressure and insulin

resistance, as well as their frequent increase in children with active JIA, the present study focuses on a CBC-derived index that incorporates platelets, mainly the SII.

The purpose of this research project is to verify the extent to which CBC derived indices, and in particular, the SII, can reflect the presence of systemic inflammation in children with obesity and JIA. For this purpose, information was retrieved from the medical records of patients admitted to one of Romania's largest pediatric hospitals, the Children's Emergency Hospital 'Louis Turcanu' in Timisoara. This process adhered to the principles outlined in the Declaration of Helsinki (1975, revised in 2013) and was approved by the Institutional Ethics Committee.

The special section of this PhD thesis aims to investigate the practical value of biomarkers derived from the complete blood count in chronic diseases with an inflammatory basis, such as obesity and juvenile idiopathic arthritis. To achieve this goal, the following objectives have been established, upon which the research directions are structured:

1. Examine the relationship between inflammatory biomarkers derived from the complete blood count, particularly the Systemic Immune-Inflammation Index, and the presence of metabolic syndrome in obese children.
2. Explore the diagnostic value of the Systemic Immune-Inflammation Index in juvenile idiopathic arthritis, as well as its predictive value by correlating it with disease severity.

## PERSONAL CONTRIBUTIONS

### **STUDY I. Assessing the relationship between Systemic immune-inflammation index and metabolic syndrome in children with obesity**

#### **Results:**

After the retrospective review of the patients electronic, 329 obese patients were identified during the period from January 2015, and February 2023. We excluded 138 patients due to concurrent active infections at the moment of hospital admission, comorbidities known to alter hematological parameters, and incomplete anthropometric or laboratory data, resulting in a study group of 191 children (110 boys, 80 girls), with a median age of 13 (IQR: 11-15) years.

Clinical data collected from the medical charts included demographic information and anthropometric measurements (weight, height, waist circumference, weight-for-height, height-for-age using national growth charts) and blood pressure. Laboratory blood tests, conducted after an 8-hour fast, included a complete blood count, glucose levels, total cholesterol, HDL-C, LDL-C levels, triglycerides and insulinemia. Also, the following CBC-derived indices were calculated, neutrophil count/lymphocyte count, platelet count/lymphocyte count, SII (platelet count  $\times$  NLR), and SIRI (neutrophil count  $\times$  monocyte count/lymphocyte count). HOMA-IR was calculated using the formula: fasting insulin [ $\mu$ U/mL]  $\times$  fasting glucose [mmol/L]/22.5 [144]. The TG:HDL-C ratio was calculated as triglycerides (mg/dL) divided by HDL-C level (mg/dL) [145], and non-HDL-C was calculated by subtracting HDL-C (mg/dL) from total cholesterol (mg/dL). According to these, patients were categorized into two groups, 66 patients diagnosed with metabolic syndrome according to IDF criteria (MetS group), and 125 without metabolic syndrome (nonMetS).

The prevalence of MetS was 34.5%. Both groups were similar regarding age and gender ( $p > 0.05$ ). In the entire study population, low HDL-C was the most prevalent component of MetS (40.8%), followed by hypertension and elevated triglycerides (both 23.5%). Hyperglycemia was the least common (16.7%). There were significantly higher values of WBC, neutrophils, and thrombocytes in the MetS group ( $p < 0.001$ ), while lymphocytes and monocytes did not show significant differences ( $p = 0.649$  and  $p = 0.112$ , respectively).

All CBC-derived indices showed a significant increase in MetS patients compared to those without MetS. The most notable difference was in SII, which was twice as high in MetS patients (711 [483.18-902.45]) compared to the non-MetS group (355.79 [277.43-467]). We conducted Spearman correlation, which proved that of the four CBC-derived indices, SII was the only one that correlated with all the cardiometabolic markers evaluated.

Additional binary logistic regression analysis to examine the association between SII and the presence of MetS. SII and TG:HDL-C proved to be the only indices significantly associated with MetS ( $p < 0.001$ ).

The diagnostic capability of SII, SIRI, HOMA-IR, TG:HDL-C and nonHDL-C in predicting MetS was also explored in this study. SII exhibited the highest discriminative ability for metabolic syndrome, with an optimal SII cut-off point of  $426 \times 10^3$  and an area under the curve, sensitivity, and specificity of 0.843, 0.83, and 0.63, respectively. ROC curve analysis revealed a similar discriminative capacity for TG:HDL-C, with an optimal cut-off point of 1.786 and a sensitivity of 0.81, although with lower specificity (0.50).

## **Study II: Evaluating the Diagnostic Performance of Systemic Immune-Inflammation Index in Childhood Inflammatory Arthritis: A Focus on Differentiating Juvenile Idiopathic Arthritis from Reactive Arthritis**

### **Results:**

The study group comprised a total of 245 children with arthritis or arthralgia. We excluded septic arthritis, Lyme arthritis, active infections, conditions known to alter hematological parameters and patients with incomplete data, resulting in a study population of 108 patients.

These patients were divided into two study groups: 70 patients with juvenile idiopathic arthritis (JIA) and 38 patients diagnosed with reactive arthritis (ReA). Subtypes of JIA were identified based on the ILAR classification criteria. Enthesitis-related arthritis (31.4%), Oligoarthritis (27.1%), and RF-negative Polyarthritis (20%) were the most prevalent subtypes within the JIA group. No significant differences were observed in terms of gender distribution between the two study groups. . Children in the reactive arthritis group tended to be younger, with a median age of 7.7



years (IQR: 3.5, 11.9), while those in the JIA group were older, with a median age of 12.1 years (IQR: 7.6, 14.5). Oligoarticular involvement was predominant in both study groups.

The JIA and ReA groups were similar in terms of white blood cells, hemoglobin, and red cell distribution width. However, JIA patients had significantly higher platelet counts ( $p<0.001$ ), neutrophils ( $p=0.046$ ), and monocytes ( $p=0.025$ ), and significantly lower eosinophils ( $p=0.049$ ). There was also a non-significant trend toward lower lymphocyte counts in JIA patients ( $p=0.090$ ). Additionally, both neutrophil-to-lymphocyte ratio (NLR) and systemic immune-inflammation index (SII) were higher in children with JIA compared to those with ReA.

Spearman correlations were used to assess relationships between the SII and the NLR with various biochemical parameters in the JIA group. Both indices displayed significant positive correlations: NLR had a strong positive correlation with CRP and a moderate positive correlation with fibrinogen. Similarly, SII showed strong positive correlations with CRP and fibrinogen, and a moderate positive correlation with ESR. In the ReA group, both the NLR and the SII had weaker positive correlations with CRP. Binary logistic regression revealed that both the NLR and the SII were initially correlated with JIA. However, after adjusting for age, only SII remained a significant independent factor associated with JIA ( $p=0.030$ ).

Receiver operating characteristic analysis showed that the SII had acceptable diagnostic accuracy for JIA, with an area under the curve (AUC) of 0.703 ( $p<0.001$ ). NLR and ESR had fair but significant discrimination capacities (AUC: 0.668 and 0.644, respectively). SII outperformed each of its component CBC parameters in discriminatory capacity.

### **Study III: Examining the Relationship between Systemic Immune-Inflammation Index and Disease Severity in Juvenile Idiopathic Arthritis**

#### **Results:**

After applying inclusion and exclusion criteria, data from 74 children diagnosed with juvenile idiopathic arthritis according to the classification criteria established by the International League of Associations for Rheumatology (ILAR) were included in this study. Patients were divided into three study groups based on their juvenile arthritis disease activity score 10 (JADAS10) score, 35.1% with low

disease activity (LDA group), 28.4% with moderate disease activity (MDA group), and 36.5% with high disease activity (HDA group). The median age of the study population was 13 years (IQR: 9-15.6), and the median disease duration was 1.2 years (IQR: 0.6-2.7). Gender distribution was not significantly different across study groups ( $p=0.136$ ). The most common ILAR subtypes were ERA (32.4%) and oligoarticular JIA (29.7%), with most patients having oligoarticular involvement (60.8%) and no significant variations between disease activity groups. As disease severity increased, all assessed disease activity parameters and biochemical inflammatory markers, particularly erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP), showed a significant increase, as expected.

The active joint count and physician and parental global assessments correspond closely with inflammatory markers. In the HDA group, the median active joint count was two, decreasing to one in MDA and zero in LDA. Similarly, physician global assessment scores decreased from seven in HDA to four in MDA and one in LDA. Parental global assessments followed a similar trend, with scores decreasing from eight in HDA to five in MDA and two in LDA. A notable progressive rise in the absolute count of white blood cells, neutrophils, and platelets was evident with increasing disease severity. A similar pattern was observed for both hematological indices, neutrophil-to-lymphocyte ratio (NLR), and systemic immune-inflammation index (SII), which increased with disease severity. Spearman correlation analysis explored links between hematological indices, disease core set variables, and JADAS10 score. Notably, NLR and SII showed significant positive correlations with all disease activity parameters, with SII demonstrating a stronger association. Both indices had the strongest correlation with CRP and the weakest with active joint count. In addition, a robust correlation was exclusively found between SII and the median JADAS10 score ( $p=0.697$ ).

Linear regression analysis was used to investigate the correlation between SII and JADAS10. Univariate analysis revealed significant associations between JADAS10 and several variables, including SII, CRP, and fibrinogen. However, in multiple linear regression analysis, only SII and CRP remained significant as independent factors associated with JADAS10.

Furthermore, we evaluated the diagnostic performance of SII in identifying high disease activity, comparing it with various hematological parameters and NLR. CRP showed the highest accuracy for high disease activity (AUC=0.841, 81%

sensitivity, and 79% specificity), followed closely by SII (AUC=0.827, 82% sensitivity, and 66% specificity). Platelet count also exhibited borderline excellent discrimination ability (AUC=0.809, 77% sensitivity, and 62% specificity), while neutrophils and NLR demonstrated acceptable discrimination ability (AUC=0.729 and AUC=0.761, respectively).

## CONCLUSIONS

The individual studies forming the basis of this doctoral thesis, representing a comprehensive effort spanning 5 years, have led to several key ideas regarding children with chronic diseases with inflammatory substrate:

The SII demonstrated diagnostic utility in identifying patients with Metabolic Syndrome within the group of obese children and showed correlations with cardio-metabolic risk biomarkers. Based on these findings, it can be hypothesized that SII, as an inflammatory biomarker, can play an additional role in measuring metabolic instability in this population. **(Paper I)**

The SII demonstrated moderate diagnostic accuracy in distinguishing patients with active JIA from those with arthritis due to other causes, specifically reactive arthritis. Given its derivation from a complete blood count, a widely available and cost-effective analysis, it can be assumed that the index can offer additional value in differentiating JIA patients, especially in situations where serology results are negative. **(Paper II)**

The SII exhibited a gradual increase corresponding to the severity of disease in children with Juvenile Idiopathic Arthritis. Furthermore, SII demonstrated an independent association with high disease activity status. In an era where there is a growing focus on exploring chemokines and other biological markers, the significance of routine blood analyses, which are widely accessible, is sometimes overlooked. Given the limitations of advanced technology in every healthcare center, the study's results underscore the complementary value of routine blood work, particularly the CBC. Since SII reflects alterations in various inflammatory cell lineages implicated in the pathogenesis of JIA, our findings advocate for considering SII as an additional tool in assessing disease activity in children with JIA. **(Paper III)**

In conclusion, the studies within this thesis suggest that the Systemic Inflammatory Index can be a valuable metric of severity, reflecting the degree of inflammation in pediatric chronic conditions such as obesity, metabolic syndrome, and juvenile idiopathic arthritis.