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PhD THESIS

**THE COMPLEXITY OF KETOACIDOSIS MANIFESTATIONS
IN TYPE 1 DIABETES IN CHILDREN AND ADOLESCENT**

A B S T R A C T

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ABSTRACT

INTRODUCTION

Type 1 diabetes (T1DM) is characterized by the chronic immune-mediated destruction of pancreatic β cells, leading to a partial or, in most cases, absolute insulin deficiency. The incidence is increasing, especially at a younger age, where a higher incidence is predicted in the coming years compared to other age groups.

Diabetic ketoacidosis (DKA) has been extensively studied, demonstrating its complexity in children and adolescents. Special attention has been given in this study to ketoacidosis at the onset of diabetes in children and adolescents. The focus in published articles has been on biological investigations conducted at the onset, correlations with the clinical status of pediatric patients in the studied group, but particularly with regard to routine analyses. Among these, the complete blood count, its fractions, lymphocyte count, erythrocyte fractions, and indices, as well as the neutrophil-lymphocyte ratio (NLR), have received special attention in recent years. The Systemic Inflammatory Response Index (SIRI) is a marker that integrates data from the components of the NLR ratio and monocytes, emerging as a valuable tool for obtaining information about the complex cellular responses associated with T1DM. These parameters have been correlated with inflammation-associated conditions such as systemic arterial hypertension, atherosclerosis, neoplasia, obesity, and diabetes mellitus along with its complications.

The state of systemic inflammation associated with diabetic ketoacidosis increases the probability of developing cerebral edema, a severe complication at the onset of diabetes mellitus, especially in the pediatric population. NLR and SIRI serves as a relevant inflammatory indicator in the context of diabetes mellitus, and its values are significant in evaluating inflammation in both chronic and acute complications of this condition.

Consequently, in collaboration with our research team, we conducted three studies that involved pediatric patients with type T1DM at the onset. We extensively analyzed some less-explored associations of diabetic ketoacidosis, focusing on biological markers, risk factors, and associated complications. These research endeavors were undertaken with the goal of obtaining additional insights into routine

investigations, emphasizing the need for a specialized diagnostic and therapeutic approach, particularly considering the accessibility of medical professionals in managing children and adolescents with type 1 diabetes mellitus at onset and ensuring continuous monitoring.

The Neutrophil-to-Lymphocyte Ratio has proven to be significantly important in predicting diabetic ketoacidosis at the onset. This finding is in line with previously published studies conducted on adult patients with type 1 diabetes mellitus, although, to our knowledge, no similar study has been reported in children. One advantage of studying children is the absence of many confounding factors that may affect NLR levels, such as associated medications and comorbidities present in adult patients with diabetes. Although there is increasing interest in inflammatory markers and their role in T1DM, there is still a significant lack of research focused on the application of SIRI in this particular context.

The purpose of this research project is to provide data on the complexity of ketoacidosis and to contribute information about the specific characteristics of ketoacidosis at the onset of type 1 diabetes mellitus in children and adolescents. These observations are made by correlating data from the observation sheets of patients admitted to Clinic 1 Pediatrics at the Emergency Clinical Hospital for Children, Louis Turcanu, Timisoara, in accordance with the principles of the Declaration of Helsinki (1975, revised in 2013) and approved by the Institutional Ethics Committee.

The thesis provides a detailed analysis of three current topics in the medical field, outlining their established goals, achieved results, and their respective significance.

Thus, the paper presents three research studies:

I. The first study focuses on the neutrophil-to-lymphocyte ratio, a marker that provides relevant details regarding the presence of diabetic ketoacidosis in children diagnosed with type 1 diabetes mellitus at onset. This indicator offers significant information that can contribute to the assessment and deeper understanding of their health status, particularly concerning the onset of this metabolic condition.

II. The second study focuses on analyzing the correlation between the neutrophil-to-lymphocyte ratio and the presence of cerebral edema in children diagnosed with severe diabetic ketoacidosis. We investigate this connection to gain a

more comprehensive perspective on the impact of NLR on the development of cerebral edema in severe cases of diabetic ketoacidosis in children. This research aims to shed light on significant aspects related to the influence of this indicator on neurological complications, thereby contributing to a better understanding of the mechanisms involved in this specific clinical situation.

III. Investigation and analysis of the role of SIRI as a potential indicator of inflammation and the occurrence of adverse events observed during the onset of T1DM in children and adolescents. Through this research, we aim to provide a detailed perspective on how SIRI can influence and offer relevant information regarding acute complications.

The specific goals to underpin the main research directions are as follows:

1. The fundamental objective of the first research was to explore and investigate the relationship between NLR and the severity of diabetic ketoacidosis in children diagnosed with type 1 diabetes at onset. We aimed to understand in greater detail how this inflammatory indicator, NLR, could influence and be associated with the severity of DKA episodes within this specific group of children diagnosed with type 1 diabetes at onset in the clinic.

2. Performing a detailed analysis of children with severe DKA, with a special focus on examining the relationship between NLR and the presence of cerebral edema. Our research is centered on developing a comprehensive understanding of the health status of this specific group of pediatric patients, aiming to gain deeper insights into how NLR may influence and be associated with the presence of cerebral edema in severe cases of diabetic ketoacidosis in children.

3. Conducting an in-depth investigation into the connection between the SIRI ratio, an inflammation marker, and acute complications in children and adolescents diagnosed with newly onset type 1 diabetes mellitus. Our research aims to provide a detailed exploration of SIRI as a prognostic factor for adverse effects associated with the progression of pediatric patients at the onset of T1DM.

4. Supplying clinical research data on pediatric patients diagnosed with Type 1 Diabetes at the onset, who are part of the registry of a reference center in western Romania, specializing in Diabetes within the Emergency Clinical Hospital for Children "Louis Turcanu," Timisoara.

PERSONAL CONTRIBUTIONS

STUDY I. The Neutrophil-to-Lymphocyte Ratio Provides Significant Information on the Degree of Diabetic Ketoacidosis in Children with Newly Diagnosed Type 1 Diabetes Mellitus

Results:

After a retrospective review of the electronic records of patients with T1D, 181 newly diagnosed children were identified during the period from January 1, 2015, to June 30, 2022. We excluded 26 patients due to concurrent acute infections, resulting in a study group of 155 children (76 boys, 79 girls), with an average age of 9.00 ± 4.39 years (range 0–18 years).

Based on the specific clinical characteristics of the disease onset, the biological evaluation included the analysis of blood samples with a focus on necessary investigations, fasting blood glucose, glycated hemoglobin (HbA1c), autoimmune antibodies, C-reactive protein (CRP), complete blood count (CBC), ketone bodies, and Astrup parameters. Patients were categorized into four groups: without DKA ($n = 35$), mild DKA ($n = 25$), moderate DKA ($n = 33$), and severe DKA ($n = 62$). There were no significant age differences among the four groups. Regarding gender, there were more female patients in the severe DKA group. There is a statistically significant difference in the parameters of the complete blood count among the four groups, particularly regarding the total leukocyte count, neutrophils, and monocytes, which showed increases with worsening DKA severity ($p < 0.0005$). Eosinophils demonstrated an inverse correlation with DKA severity ($p < 0.001$), decreasing as DKA became more severe. Lymphocytes recorded statistically lower values in patients with severe DKA compared to those with mild and moderate DKA.

Statistically, a Kruskal–Wallis H test was conducted to determine if there were significant differences in NLR scores between children without ketoacidosis and those with mild, moderate, or severe ketoacidosis. This analysis revealed significant statistical differences in median NLR scores between those with severe DKA and those with moderate DKA but not between moderate DKA and no DKA. The glycated hemoglobin was approximately equal across the four groups (mean = 11.40 ± 2.01).

We conducted a multiple regression analysis to examine the relationship between blood pH and variables such as age, gender, HbA1c, PCR, and NLR. The

resulting model from this analysis revealed a significant association with blood pH ($p < 0.001$). Specifically, NLR score and age made substantial contributions to this association, with $p < 0.001$.

NLR combines the predictive power of both increased neutrophil and decreased lymphocyte counts, has the advantages of being ubiquitous, and more stable compared to the absolute count. Results from the present study, the WBC count and the NLR were found to be higher in patients with DKA.

Study II: The Correlation Between the Neutrophil-to-Lymphocyte Ratio and the Incidence of Cerebral Edema in Pediatric Patients with Severe Diabetic Ketoacidosis

Results:

The study group included a total of 98 children with severe DKA, of which 86 had T1D at onset, and 12 were previously diagnosed cases. Children were divided into two categories: conscious (28 patients), with subclinical cerebral edema (59 patients), and overt cerebral edema (11 patients). The median age of the entire study cohort was 9.7 [interquartile range (IQR): 11–15] years, with no significant differences between the study groups, although patients with altered mental status tended to be younger. Additionally, a trend toward female predominance was observed with worsening neurological impairment, although it did not reach statistical significance ($p = 0.186$). Furthermore, blood pressure increased significantly with the worsening of neurological impairment. As expected, children with cerebral edema had a longer stay in the pediatric intensive care unit and a longer time until acidosis correction ($p < 0.001$).

Patients with signs of cerebral edema demonstrated more pronounced metabolic acidosis ($p < 0.001$), elevated corrected sodium levels ($p = 0.019$), osmolarity ($p = 0.005$), and blood urea nitrogen ($p = 0.002$). The study groups exhibited similar mean concentrations of glucose, HbA1c, insulinemia, and CRP, although a trend towards a gradual decrease in plasma insulin and C-peptide levels was observed with worsening neurological impairment.

Progressively, the median NLR scores increased from those without neurological impairment (2.82) to those with subclinical and overt cerebral edema (5.66 and 8.60, respectively). Subsequently, the statistical analysis highlighted significant differences in median NLR scores between those in an alert state and those

with subclinical cerebral edema ($p < 0.001$) and clinically evident cerebral edema ($p < 0.001$). However, median NLR scores were similar between those with subclinical and clinically evident cerebral edema ($p = 0.292$).

Reduced blood pH content and elevated levels of NLR and blood urea were correlated with the presence of cerebral edema ($p < 0.001$); we also observed a weak correlation between the presence and severity of cerebral edema and elevated corrected sodium levels and associated co-infection. Regression analysis was used to investigate the independence of NLR in predicting the presence of cerebral edema. After adjusting for multifactorial risk for potential confounders such as age, pH, corrected sodium, and blood urea nitrogen, NLR remained positively associated with cerebral edema ($p = 0.045$).

Study III: The Systemic Inflammatory Response Index (SIRI) as an Indicator for Predicting Adverse Outcomes in Children Newly Diagnosed with Type 1 Diabetes Mellitus

Results:

According to the inclusion and exclusion criteria, data from 186 children aged 1 to 18 years diagnosed with T1DM were included in this study. Patients were divided into three groups based on SIRI tertiles. The study population had a median age of 9.4 years (IQR: 5.1–12.7 years). Gender distribution did not exhibit significant variances across study groups ($p = 0.267$). Patients with elevated SIRI values were more frequently female and tended to exhibit heightened disease activity. This correlated with prolonged intensive care unit (ICU) admissions, increased incidence of complications, and a greater need for mechanical ventilation. Concerning laboratory parameters, patients with elevated SIRI values demonstrated a notable increase in WBCs, neutrophils, monocytes, platelets, and CRP, alongside a significant decrease in lymphocytes and eosinophils. Furthermore, patients with elevated SIRI values were more inclined to have lower venous pH and C-peptide levels. HbA1c values remained consistent across all SIRI tertiles.

When categorizing patients based on their co-infection status, we noted no statistically significant variances between the median SIRI values of patients with co-infection (7, IQR: 3.36–11.73) upon admission and those without co-infection (9.96, IQR: 5.18–20.51).

The connection between median SIRI values and adverse events encountered during hospitalization for the onset of T1DM was investigated through Spearman correlation analysis. The most notable associations between SIRI and adverse events during the hospital stay were identified with prolonged ICU length of stay ($p = 0.606$) and acute kidney injury ($p = 0.602$). Moreover, mean SIRI values exhibited significant correlations with evident cerebral edema ($p = 0.296$) and sepsis ($p = 0.272$). Similar correlations were observed regarding venous pH. When examining glucose metabolism markers, only C-peptide demonstrated a significant correlation with unfavorable events during the hospital stay, albeit to a lesser degree compared to SIRI and venous pH.

Multivariate logistic regression, employing a stepwise selection approach, revealed that SIRI maintained its significant correlation with acute complications and prolonged time in ICU, even when specifically examining patients diagnosed with severe DKA.

In our retrospective analysis of data collected over a span of ten years from patients experiencing new-onset T1DM, a notable correlation emerged between elevated SIRI values and the occurrence of adverse events during hospital admission. This association is likely attributable to the severity of DKA, a common complication accompanying T1DM onset. DKA is known to predispose individuals to a range of complications and induces alterations in the CBC profile of affected patients. These changes in CBC parameters may contribute to the higher SIRI values observed in patients experiencing adverse events during hospitalization.

CONCLUSIONS

Diabetic ketoacidosis is a severe acute complication that primarily occurs at the onset of type 1 diabetes, with an incidence ranging from 13% to 80%. This condition is characterized by systemic inflammation, and inflammatory markers such as blood leukocytes and PCR play an essential role in its development. Although the blood hemoleucogram analysis is a common part of assessing diabetic patients, the leukocyte fractions have not received significant attention from diabetes specialists in the past. However, in recent years, there has been an increased interest in NLR and SIRI as markers of systemic inflammation in various conditions, including cardiac

diseases, neoplasms, and obesity, as well as complications related to diabetes, such as diabetic foot ulcers and retinopathy. In this context, our objective was to investigate the association between NLR and SIRI in the severity of diabetic ketoacidosis in children with type 1 diabetes at the onset, as well as the correlations between NLR and the occurrence of acute complications.

This paper provides additional insights into laboratory analyses for children with DKA at the onset of T1D. It emphasizes that higher levels of NLR are associated with an increased frequency of DKA in children with T1D at onset and positively correlate with the severity of DKA. To date, this is the first research assessing NLR based on the severity of DKA in children with T1D at onset.

This finding holds clinical significance, especially in smaller hospitals where blood gas analysis is not a common practice, and it could contribute to improving early diagnosis of DKA in children with T1D at onset, thereby facilitating appropriate care based on clinical severity.

The results of the second study indicate that NLR could be a useful additional tool in identifying patients at high risk of developing severe cerebral edema. Therefore, more intensive neurological monitoring might be necessary for the prompt detection of initial warning signs and the rapid initiation of specific treatment.

In this context, the first study presented in the doctoral thesis is the first study conducted at the pediatric age in the specialized literature. Patients with high SIRI values had an increased risk of adverse events during hospitalization for the onset of T1DM. This provides a new perspective on risk assessment, potentially leading to early intervention strategies by using SIRI as a predictive marker. Additionally, this practical tool allows clinicians in minimally equipped health units to identify high-risk pediatric patients who require admission to medical units equipped with an intensive care unit. Patients who initially present in the outpatient clinic, with minimal routine investigations and who show clinical signs of diabetes, high levels of glucose and high SIRI values, would benefit from a prompt referral to a hospital equipped with an intensive care unit.

It has also been mentioned that NLR and SIRI superiority over the total leukocyte count, but the consequences of this increased imbalance and the characteristics of leukocyte release in hyperglycemic emergency situations require further investigation.

Therefore, our objective was to assess changes in the total and differential leukocyte counts and identify their significance in reflecting the severity of DKA, emphasizing the importance of complete blood analysis in the progression of diabetes mellitus in children and adolescents.

Moreover, to the best of our knowledge, this doctoral thesis represents the first attempt to analyze the significance of this simple hematologic parameter in determining the severity of DKA, acute complications such cerebral edema in pediatric patients.

In support of this perspective, the predictive values of NLR and SIRI are comparable to various other inflammatory markers, such as CRP, tumor necrosis factor-alpha and interleukin-6, in detecting subclinical inflammation and endothelial dysfunction in various clinical studies.

In conclusion, this study provides valuable insights into the presence of DKA in children with T1D at onset, its correlation with the most common acute complications including cerebral edema, acute renal failure, and electrolyte imbalances. This thesis demonstrates the importance of NLR and SIRI as readily obtainable inflammatory markers in clinical practice and their predictive value for acute complications in children and adolescents with T1D.