ORIGINAL ARTICLE

GLYCEMIA, TEMPERATURE, AND HEMATOLOGICAL VARIABLES AS BIOMARKERS IN A MOUSE MODEL OF EXPERIMENTAL SEPSIS

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Highlights: (1) Hypothermia and decreased glycemia may indicate early diagnosis of sepsis. (2) Hematological parameters: MCV, platelets, and NLPR monitor and predict sepsis severity. (3) Hypothermia, glycemia, and hematological variables are crucial for the diagnosis and monitoring of sepsis.

http://dx.doi.org/10.21527/2176-7114.2026.51.15465

How to cite:
Knebel SC, Sulzbacher LM, Sulzbacher MM, Fiorin PBG, Ludwig MS, de Oliveira VA. et al. Glycemia, temperature, and hematological variables as biomarkers in a mouse model of experimental sepsis. Rev. Contexto & Saúde. 2026;26(51):e15465

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GLYCEMIA, TEMPERATURE, AND HEMATOLOGICAL VARIABLES AS BIOMARKERS IN A MOUSE MODEL OF EXPERIMENTAL SEPSIS

ABSTRACT

Sepsis is a life-threatening condition characterized by infection and widespread inflammation, resulting in multiple organ dysfunction syndrome (MODS). This study aimed to evaluate whether temperature, body weight, glycemia, hematological parameters, and their ratios can be used as biomarkers in a mouse model of experimental sepsis. An experimental animal study using mice was conducted, divided into two groups: Control group (CTRL) and Sepsis group (SEP). Animals in the control group received 0.9% saline solution, whereas those in the sepsis group received an intraperitoneal injection of 20% fecal solution (1 mg/g). Clinical and laboratory biomarkers were assessed at 0, 4, 12, and 24 hours throughout the experimental protocol. It was demonstrated that, as early as 4 hours after sepsis induction, hypothermia and decreased blood glucose levels were observed in the sepsis group compared to the control group. At 24 hours, the sepsis group showed reductions in mean corpuscular volume (MCV) and platelet counts. In addition, an increase in the neutrophil-to-lymphocyte and platelet ratio (NLPR) was identified. Thus, these findings demonstrate that hypothermia, glycemia, and hematological parameters may serve as important biomarkers for the diagnosis and monitoring of sepsis.

Keywords: Sepsis; Clinical Markers; Hematology; Mouse Model.

INTRODUCTION

Sepsis is a life-threatening condition characterized by infection and widespread inflammation, resulting in dysfunction of multiple organs. It is a life-threatening organ dysfunction caused by a dysregulated host response to infection and is diagnosed based on an increase of two or more points on the Sequential Organ Failure Assessment (SOFA) score [(1)], indicating dysfunction in the cardiovascular, hepatic, renal, respiratory, neurological, or coagulation systems. Undoubtedly, the conceptualization and prevention strategies for sepsis are designed to deepen the understanding of the severity of the infectious condition, its progression, and its characterization, which are essential for early diagnosis and appropriate clinical management.

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According to estimates from the World Health Organization (WHO), sepsis affects approximately 49 million people every year, resulting in nearly 11 million deaths. Among the main infectious foci that trigger sepsis, abdominal infections are particularly prominent [(2)]. Moreover, there has been an increasing incidence of sepsis, particularly evident among elderly individuals and those with comorbidities. Conservative estimates indicate that sepsis is one of the leading causes of mortality and severe illness worldwide [(3)].

According to the *Instituto Latino-Americano de Sepse* (ILAS, as per its Portuguese acronym), approximately 240,000 deaths due to sepsis occur annually in Brazilian Intensive Care Units (ICUs), highlighting the need to implement predictive biomarkers to enhance the health system's capacity for early anticipation, diagnosis, and intervention in severe sepsis [(4)].

From this perspective, septic patients often present a linear evolution of clinical manifestations, beginning with systemic inflammatory response syndrome (SIRS) and potentially progressing to clinical and laboratory deterioration and, in severe cases, septic shock. However, there remain substantial gaps in biomarkers capable of predicting outcomes and prognoses [(5)]. The integration of clinical and laboratory data is highly advantageous, given its capacity to improve predictions of progression and mortality in septic patients, offering valuable evidence for anticipating or determining future outcomes.

Accurate analysis and interpretation of clinical and laboratory biomarkers play a crucial role in evaluating, stratifying, and monitoring patients' health status, thereby significantly contributing to improved prognosis. Among the various laboratory biomarkers, hematological parameters and their derived ratios stand out for their high sensitivity and specificity in sepsis screening, as well as their relatively low cost, broad applicability, and rapid turnaround time. These biomarkers demonstrate sensitivity in the early stages of sepsis and can detect physiological responses that aid both in diagnosis and in monitoring disease progression and outcomes.

Furthermore, clinical biomarkers, such as temperature and body weight, together with glycemia (a laboratory biomarker), also play important roles in assessing health status and predicting outcomes in various conditions. Glycemia is a crucial indicator for diagnosing and monitoring metabolic status; body temperature reflects changes that may indicate infections or disease; and body weight serves as a marker of nutritional status and energy balance. Like sepsis

biomarkers, these variables provide precise information for early diagnosis, monitoring disease progression, and predicting clinical outcomes.

According to the latest ILAS national report (2024) [4], the mean age of individuals affected by sepsis in Brazil is approximately 65 years. Sepsis tends to present more severely in elderly populations, resulting in higher mortality rates, likely due to chronic non-communicable diseases (NCDs) that affect cardiovascular, respiratory, and metabolic functions. Therefore, monitoring hematological variables, including leukogram changes (reflecting increases or decreases in the inflammatory response) and reductions in circulating platelet counts, is a sensitive approach for detecting microbial infections with systemic dysfunction. Additionally, assessing leukocyte–platelet ratios provides valuable insights for monitoring acute and severe inflammatory processes, including sepsis [6].

Thus, the present study aims to evaluate whether temperature, body weight, glycemia, hematological parameters, and their ratios may serve as biomarkers in an experimental sepsis model.

MATERIALS AND METHODS

Study Design

This is an *in vivo* experimental study using a C57BL/6 mouse model.

Animals

The study was approved by the Ethics Committee for the Use of Animals (CEUA) of the Regional University of Northwestern Rio Grande do Sul (UNIJUÍ) under Protocol No. 008/21 (Appendix A). A total of 32 C57BL/6 mice, both male and female, approximately 210 days old, obtained from the UNIJUÍ Animal Facility, were used.

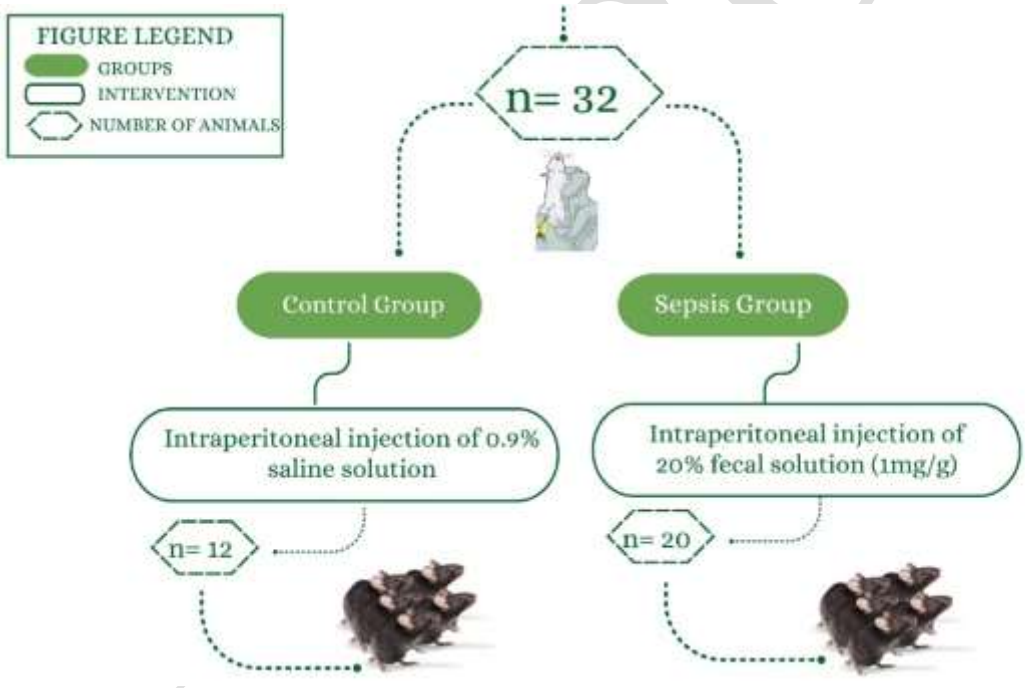
The animals were housed in cages under controlled temperature conditions (24 ± 2 °C) and a 12-hour light/dark cycle. All experimental groups received standardized laboratory rodent

feed (Nuvilab CR-1), provided in feeders placed on the cage lids, and potable water *ad libitum*. The study followed the ethical principles established by the Brazilian Guidelines for the Care and Use of Animals in Teaching or Scientific Research Activities [7].

Experimental Design

The animals were divided into two experimental groups: Control (CONT, n = 12) and Sepsis (SEP, n = 20), as illustrated in Figure 1. At the beginning of the experiment (time 0), a 20% fecal solution was administered to induce sepsis in animals in the SEP group, whereas the CONT group received physiological saline solution.

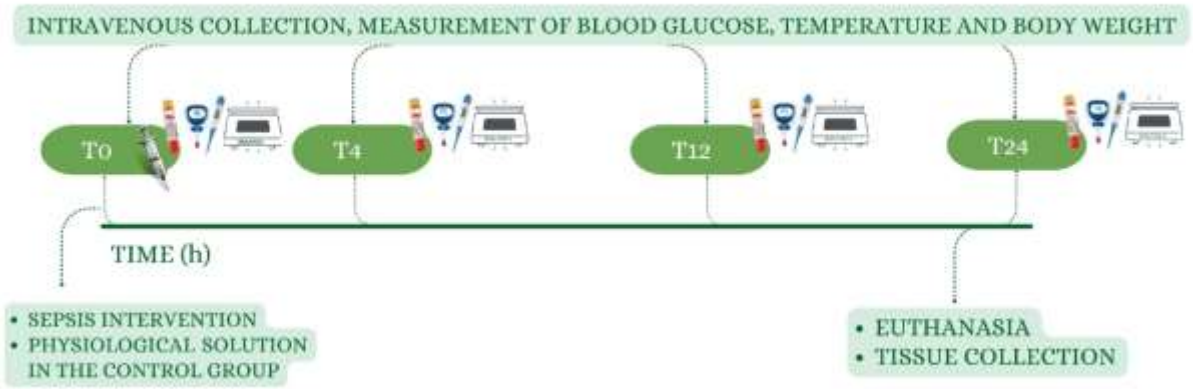
Figure 1 – Experimental design of the study: Control (CONT) and Sepsis (SEP).



Source: Prepared by the authors.

The animals were evaluated for glycemia, body weight, complete blood count (CBC), and temperature at baseline (time 0) and at 4, 12, and 24 hours after sepsis induction. Subsequently, the animals were euthanized for blood collection and subsequent determination of hematological parameters, as illustrated in Figure 2.

Figure 2 – Chronological outline of sepsis induction and intervention from 0 to 24 hours.



Source: Prepared by the authors.

Experimental Sepsis Protocol

Animals subjected to sepsis received a 20% fecal solution (200 mg/mL) via intraperitoneal injection at a dose of 1 mg/g, whereas animals in the control group received 0.9% physiological saline (5 µL/g) [8]. Peritonitis is an alternative approach for establishing a condition of generalized infection [9] and mimicking a severe sepsis scenario [9]. This sepsis model, involving intraperitoneal administration of an autogenous fecal solution, simulates an infection caused by the host's own commensal bacteria and induces a severe status of systemic infection [8,10,11].

Hematological Analyses

Blood samples were collected in tubes containing anticoagulant (EDTA) for the determination of hematological parameters (5 µL of EDTA per 500 µL of blood). Automated measurements were performed using the ABX Micros 60 hematology analyzer (Horiba), according to the manufacturer's recommendations. The following parameters were obtained:

- Erythrogram: red blood cell count (RBC), hematocrit (HCT), mean corpuscular volume (MCV), red cell distribution width (RDW), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC).
- Leukogram: total leukocyte count (TLC) or white blood cell count (WBC), absolute neutrophil count (#NEUT), eosinophils (#EOS), basophils (#BASO), lymphocytes (#LYMPH), and monocytes (#MON).
- Platelet profile: platelet count (PLT) [12].

For hematological analyses, samples were diluted 1:3 in 0.9% physiological saline, and all measurements were performed in triplicate. Subsequently, blood smears were prepared on glass slides, stained with Giemsa and May-Grünwald (Newprov), and evaluated by an experienced professional to confirm the automated data.

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For the determination of hematological ratios, the formulas described below were applied.

AISI: Aggregate Index of Systemic Inflammation. # Absolute Count.

$$AISI = \frac{^{\#}\text{NEUTROPHILS} * ^{\#}\text{MONOCYTES} * ^{\#}\text{PLATELETS}}{^{\#}\text{LYMPHOCYTES}}$$

MLR: Monocyte-to-Lymphocyte Ratio. # Absolute Count.

$$MLR = \frac{^{\#}\text{MONOCYTES}}{^{\#}\text{LYMPHOCYTES}}$$

NLPR: Neutrophil-to-Lymphocyte and Platelet Ratio. # Absolute Count.

$$NLPR = \frac{^{\#}\text{NEUTROPHILS} * 100}{^{\#}\text{LYMPHOCYTES} * ^{\#}\text{PLATELETS}}$$

NLR: Neutrophil-to-Lymphocyte Ratio. # Absolute Count.

$$RNL = \frac{^{\#}\text{NEUTROPHILS}}{^{\#}\text{LYMPHOCYTES}}$$

PLR: Platelet-to-Lymphocyte Ratio. # Absolute Count.

$$RPL = \frac{^{\#}\text{PLATELETS}}{^{\#}\text{LYMPHOCYTES}}$$

SII: Systemic Immune-Inflammation Index. # Absolute Count.

$$SII = \frac{^{\#}\text{NEUTROPHILS} * ^{\#}\text{PLATELETS}}{^{\#}\text{LYMPHOCYTES}}$$

SIRI: Systemic Inflammation Response Index. # Absolute Count.

$$SIRI = \frac{^{\#}\text{NEUTROPHILS} * ^{\#}\text{MONOCYTES}}{^{\#}\text{LYMPHOCYTES}}$$

Blood Glucose, Rectal Temperature, and Body Weight Measurements

To obtain blood glucose values, measurements were performed by puncture of the distal portion of the tail (~5 μL), using an Optium Xceed® glucometer. Rectal temperature was measured with a digital thermometer, and body weight was recorded using a semi-analytical balance.

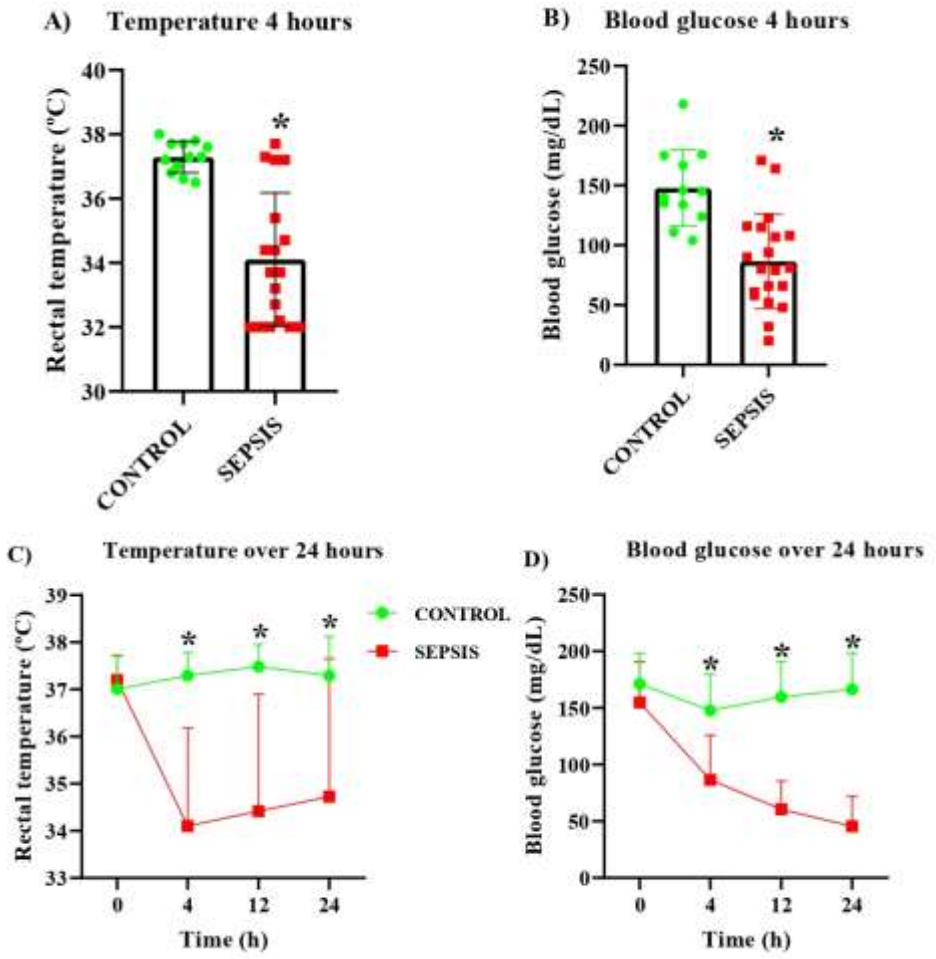
Statistical Analyses

Data were analyzed using the GraphPad Prism 8 statistical software. Normality was assessed using the Shapiro–Wilk test, followed by an outlier test, and results were expressed as measures of central tendency (mean) and dispersion (standard deviation). Variables measured over time were compared between groups using two-way ANOVA (time × intervention). Parameters that did not involve repeated measures were tested for normality and analyzed with the appropriate test (Student's t-test or Mann–Whitney test). The receiver operating characteristic (ROC) curve was constructed based on the predicted probability value. A p-value < 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

The results showed a decrease in temperature and glycemia as early as 4 hours after sepsis induction, indicating that animals in the sepsis group developed hypothermia and reduced glycemia within a short period of time. This demonstrates that both the clinical parameter (temperature) and the laboratory parameter (glycemia) showed sensitivity in identifying sepsis.

Figure 3 – Clinical and laboratory parameters at 4 hours (A and B) and over 24 hours (C and D) after induction.



Legend: Control (n = 12) and Sepsis (n = 20), where $p < 0.05$.
Source: Prepared by the authors.

Regarding temperature, as shown in Figure 3A, sepsis led to hypothermia 4 hours after induction, which often represents a response of the body's thermoregulatory system to sepsis resulting from an uncontrolled inflammatory response. The notable alteration in thermal balance caused by dysregulated inflammation highlights the importance of early temperature monitoring during this period for the effective prediction of sepsis.

According to Zhao and Zhang (2024), body temperature is an effective marker for assessing disease severity, specifically in sepsis [12]. Some studies have reported that septic patients may present impaired central nervous system function, affecting the balance of the hypothalamic–pituitary axis and, consequently, impairing the secretion of neurohypophysial hormones, such as vasopressin [13,14,15]. This hormonal imbalance impacts the body's ability to regulate blood pressure and may play a role in the occurrence of hypothermia, since effective vasoconstriction, which is essential for maintaining adequate body temperature, may be compromised by hormonal dysfunction.

Research has shown that hypothermia may serve as a predictor of mortality in non-elderly patients with sepsis. The relationship between hypothermia and adverse outcomes, such as death, reinforces the need for a predictive approach in clinical practice and individualized recognition of patient characteristics, utilizing clinical aspects, such as temperature, to support a rapid and accurate diagnosis of sepsis [16].

Furthermore, a subsequent study assessed the impact of abnormal body temperature on the severity and outcomes of septic patients, concluding that hypothermia ≤ 36.5 °C was associated with increased mortality and organ failure, regardless of the presence of septic shock. These findings underscore the critical relevance of temperature monitoring and thermal regulation in the clinical progression of septic patients. Additionally, they reinforce hypothermia as a relevant prognostic factor, both for diagnosis and for predicting the worsening of sepsis [17].

As shown in Figure 3B, the decrease in glycemia in the septic group at 4 hours after induction is clinically relevant, and this laboratory biomarker remained at lower levels throughout the 24-hour experiment (Figure 3D). Previous investigations have reported that reduced glycemia and hypoglycemia frequently occur in septic patients. Moreover, some

authors have described that, in the early stages of sepsis, the occurrence of hypoglycemia is associated with increased disease severity, higher mortality, and prolonged hospitalization [18]. Another study has associated hypoglycemia with increased severity and high mortality in patients with severe sepsis, reinforcing that septic patients with hypoglycemia have an elevated risk of death [19]. Thus, early hypoglycemia in sepsis is important for both diagnosis and prognosis, reflecting the rapid progression of the condition and its direct implications for predicting worsening and survival.

In relation to Figure 3, temperature (A) and glycemia (B) analyses show that at 4 hours, the sepsis group already presented decreased glycemia and hypothermia, and over the 24-hour period (Figures 3C and 3D), these parameters remained significantly altered in the sepsis group compared with controls. Studies have reported that hypothermia at ICU admission can predict hospital mortality [16], and likewise, septic patients with hypoglycemia have a slightly increased risk of hospital mortality [20]. Considering early treatment in septic patients presenting hypothermia and/or hypoglycemia at the onset of monitoring, this approach may offer a promising strategy for reducing hospital mortality

When assessing glycemia and temperature over the 24-hour experimental period (Table 1), it was observed that glycemia remained significantly altered in septic animals at 4, 12, and 24 hours after induction, indicating its importance not only for diagnosis but also for monitoring and prognostic assessment of sepsis. In contrast to glycemia, temperature was reduced in the sepsis group at 4 and 12 hours, indicating a clinical picture of hypothermia. In contrast, no significant differences were observed for body weight.

In this context, hypothermia at 4 and 12 hours after induction may occur as a consequence of reduced cardiac function and impaired maintenance of blood pressure, as observed in experimental models of sepsis induced by peritonitis, leading to decreased blood pressure and, consequently, hypothermia [9,10,21]. Peritonitis affects cells and tissues, weakening blood vessels and potentially reducing circulating blood volume as plasma extravasates from the intravascular space. This leads to cardiovascular dysfunction [21], which may ultimately result in hypothermia. These mechanisms can also be observed in patients, emphasizing the need for a comprehensive understanding and effective interventions.

Previous research highlighted the frequency of renal, cardiac, hepatic, and bacteremia-related complications in hypoglycemic patients. In their analysis of 48 septic patients with hypoglycemia and subsequent ICU-acquired complications, 31 experienced mild hypoglycemic events prior to the onset of complications, thereby predicting risk [22]. This study demonstrates the complications associated with hypoglycemia and suggests that glucose levels may assist in predicting complications in septic patients. In this study, this finding aligns with significant alterations detected as early as 4, 12, and 24 hours after sepsis induction.

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Table 1 – Clinical and laboratory parameters expressed as measures of central tendency (mean), dispersion (standard deviation), and p-values for the period from 0 to 24 hours of the experiment.

PARAMETERS	0 HOURS		p-value	4 HOURS		P Value	12 HOURS		p-value	24 HOURS		p-value
	CONTROL	SEPSIS		CONTROL	SEPSIS		CONTROL	SEPSIS		CONTROL	SEPSIS	
	Mean and Standard Deviation			Mean and Standard Deviation			Mean and Standard Deviation			Mean and Standard Deviation		
GLYCEMIA (mg/dL)	171.1 ± 27.05	154.9 ± 35.72	0.1855	147.9 ± 31.76	86.55 ± 39.48	**<0.0001***	159.8 ± 30.92	60.45 ± 25.15	**<0.0001***	166.4 ± 31.96	45.50 ± 26.45	**<0.0001***
TEMPERATURE (°C)	37.01 ± 0.706	37.20 ± 0.527	0.3879	37.29 ± 0.489	34.10 ± 2.078	**0.0001***	37.48 ± 0.471	34.42 ± 2.482	**0.0022***	37.29 ± 0.828	34.73 ± 2.925	0.0842
BODY WEIGHT (g)	30.21 ± 5.662	29.68 ± 4.371	>0.9999	29.52 ± 5.504	29.37 ± 4.264	0.9301	28.83 ± 5.409	28.97 ± 4.267	0.833	28.78 ± 5.222	28.40 ± 4.839	0.8474

Legend: p-value: $p < 0.05$.

Source: Prepared by the authors**.**

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Table 2 – Association between hematological parameters and their ratios expressed as measures of central tendency (mean), dispersion (standard deviation), and p-value at 24 hours of the experiment.

PARAMETERS	24 HOURS		p-value
	CONTROL	SEPSIS	
	Mean and Standard Deviation		
RBC ($x10^6/mm^3$)	6.307 ± 1.345	7.013 ± 2.328	0.4419
HGB (g/dL)	9.767 ± 1.538	10.53 ± 3.043	0.5095
HCT (%)	28.50 ± 6.203	30.70 ± 10.78	0.6029
MCV (μ^3)	45.18 ± 1.762	43.59 ± 1.261	**0.0266***
RDW (%)	16.18 ± 1.325	15.96 ± 1.482	0.7417
MCH (pg)	15.70 ± 1.465	14.56 ± 0.6844	0.0554
MCHC (%)	34.78 ± 3.679	33.35 ± 2.084	0.3492
WBC (mm^3)	4.567 ± 1.817	3.060 ± 1.826	0.0897
#NEUT (mm^3)	793.3 ± 544.1	727.0 ± 443.4	0.7804
#MON (mm^3)	112.3 ± 117.7	19.00 ± 22.26	0.0802
#LINF (mm^3)	3.612 ± 1.352	2.327 ± 1.486	0.073
NLR (mm^3/ mm^3)	0.203 ± 0.109	0.377 ± 0.266	0.1135
LMR (mm^3/ mm^3)	0.030 ± 0.030	0.033 ± 0.037	0.7811
LPR (mm^3/ mm^3)	0.300 ± 0.092	0.519 ± 0.399	0.1285
NLPR (mm^3/ mm^3/ mm^3)	0.020 ± 0.010	0.040 ± 0.013	**0.0028***
SII (mm^3/ mm^3/ mm^3)	211.8 ± 119.4	290.0 ± 213.7	0.3521
AISI (mm^3/ mm^3/ mm^3/ mm^3)	31.081 ± 25,81	25.564 ± 28.40	0.7103
SIRI (mm^3/ mm^3/ mm^3)	28.25 ± 23.05	34.71 ± 37.72	0.7058
PLT ($x10^3/mm^3$)	1.077 ± 97.48	763.8 ± 190.3	**0.0007***

Legend: *P < 0.05; RBC - Red Blood Cells; HGB - Hemoglobin; HCT - Hematocrit; MCV - Mean Corpuscular Volume; RDW - Red Blood Cell Distribution Width; MCH - Mean Corpuscular Hemoglobin; MCHC - Mean Corpuscular Hemoglobin Concentration; WBC - White Blood Cells; #NEUT - Neutrophils; #MON - Monocytes; #LYMPH - Lymphocytes; NLR - Neutrophil-to-Lymphocyte Ratio; MLR - Monocyte-to-Lymphocyte Ratio; PLR - Platelet-to-Lymphocyte Ratio; NLPR - Neutrophil-to-Lymphocyte and Platelet Ratio; SII - Systemic Inflammation Index, (Neutrophils × Platelets) / Lymphocytes; AISI - Aggregate Index of Systemic Inflammation, (Neutrophils × Monocytes × Platelets) / Lymphocytes; SIRI - Systemic Inflammation Response Index, (Neutrophils × Monocytes) / Lymphocytes; PLT - Platelets.

Source: Prepared by the authors**.**

In the laboratory assessment of the complete blood count (Table 2), it was observed that the Mean Corpuscular Volume (MCV) decreased in the sepsis group 24 hours after induction. Regarding leukocyte ratios, an increase in the neutrophil-to-lymphocyte and platelet ratio (NLPR) was identified at the 24-hour time point. Additionally, in the platelet profile, there was also a reduction in platelet count in the animals in the sepsis group.

When hematological parameters were analyzed in a study including 10 healthy volunteers and 15 patients with severe sepsis or septic shock, an increase in MCV was observed in the septic group compared with the control group [22]. However, in this study, a decrease in MCV was observed 24 hours after induction, likely reflecting the pathophysiology of sepsis, which causes dysregulated inflammation and alterations in erythrocyte volume and morphology associated with intense inflammatory responses and microangiopathies [24]. The reduction in MCV indicates erythrocyte injury resulting from hypotension, vasodilation, and endothelial alterations, demonstrating that changes in erythrocyte size are associated with the pathological process of sepsis, as well as with complications, highlighting their role as biomarkers for monitoring septic patients.

In infectious processes, neutrophil count increases as the inflammatory disease progresses, as they are produced in the bone marrow and released into the bloodstream to combat infections and inflammation [25], including sepsis. Similarly, lymphocyte count reflects the patient's immune status, helping to defend the body against infections and diseases by recognizing and responding to specific pathogens, thereby multiplying to produce cells that fight infection. Platelets, in turn, play a key role in hemostasis and contribute to the innate immune response during inflammation [26].

NLPR combines these inflammatory indicators to predict risk factors. In this study, this ratio was significantly increased 24 hours after induction in the sepsis group compared with controls, indicating early prognostic value for disease progression. Analyses show that NLPR values on days 1, 3, and 5 of ICU admission correlate with in-hospital mortality among septic patients [27]. Accordingly, in this study, predictive 24-hour NLPR data in the sepsis group showed a significant increase. However, it did not show the early sensitivity observed for hypothermia and decreased glycemia at just 4 hours after induction.

Thus, it can be observed that platelets played a fundamental role in the evaluation of the response, given their crucial function in blood coagulation. At 24 hours after induction, platelet levels showed significant reductions in the sepsis group compared with the control group. Previous studies have demonstrated that a common disorder in sepsis and acute infections is platelet reduction, often leading to acute thrombocytopenia [28].

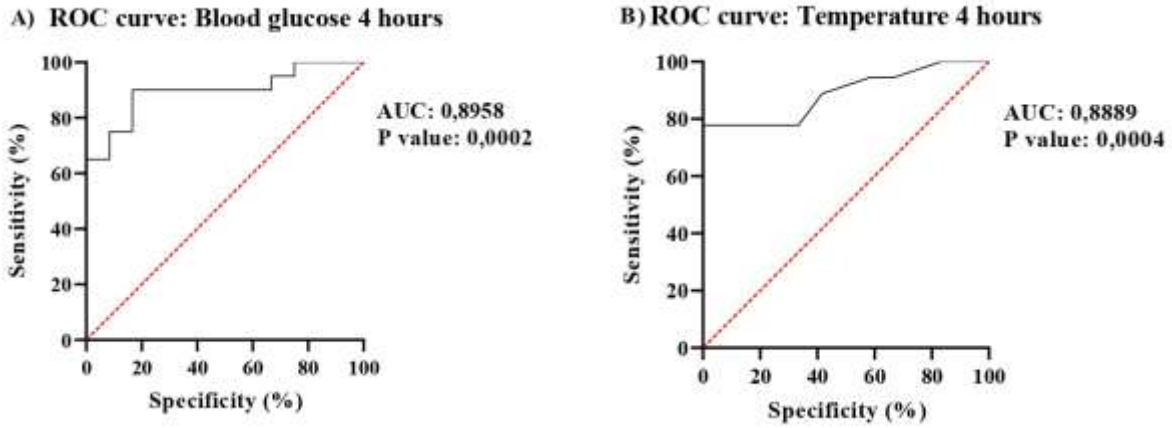
Studies report that platelet counts provide a reliable assessment for diagnosing septic shock during the first week of ICU admission, and that a decrease in platelet count during the first five days of ICU hospitalization is associated with an increased risk of mortality [29]. In this research, a notable decrease in platelet count was observed within a short period, particularly during the first 24 hours after sepsis induction. This relevant observation may represent an early sign of changes in the health status of septic patients. A marked drop in platelet count during this period may alert clinicians to the possibility of septic shock or other severe conditions.

Some diseases, including sepsis, can lead to disseminated intravascular coagulation (DIC) due to pathological activation of hemostasis as part of a systemic inflammatory response. In this condition, sepsis leads to abnormally high levels of tissue factor in the bloodstream, which trigger cascade reactions resulting in DIC. There is also functional deficiency of activated protein C, which plays an important role in controlling the coagulation process, indicating sustained activation of the coagulation system [30].

The association between sepsis and DIC is well recognized, as this condition involves dysregulated coagulation with thrombus formation, which can impair blood flow in small vessels and worsen the disease. Consequently, DIC is characterized by diffuse activation of intravascular coagulation, leading to the formation and deposition of fibrin in the microvasculature, which can cause vascular occlusion, compromise blood flow to organs and, together with other alterations, contribute to multiple organ failure [30].

Our results emphasize the importance of continuous monitoring of hematological parameters. Monitoring changes in MCV, platelet count, and NLPR may be crucial for improving prognosis and ensuring effective treatment of septic patients.

Figure 4 – ROC curve after 4 hours of induction, glycemia and temperature.

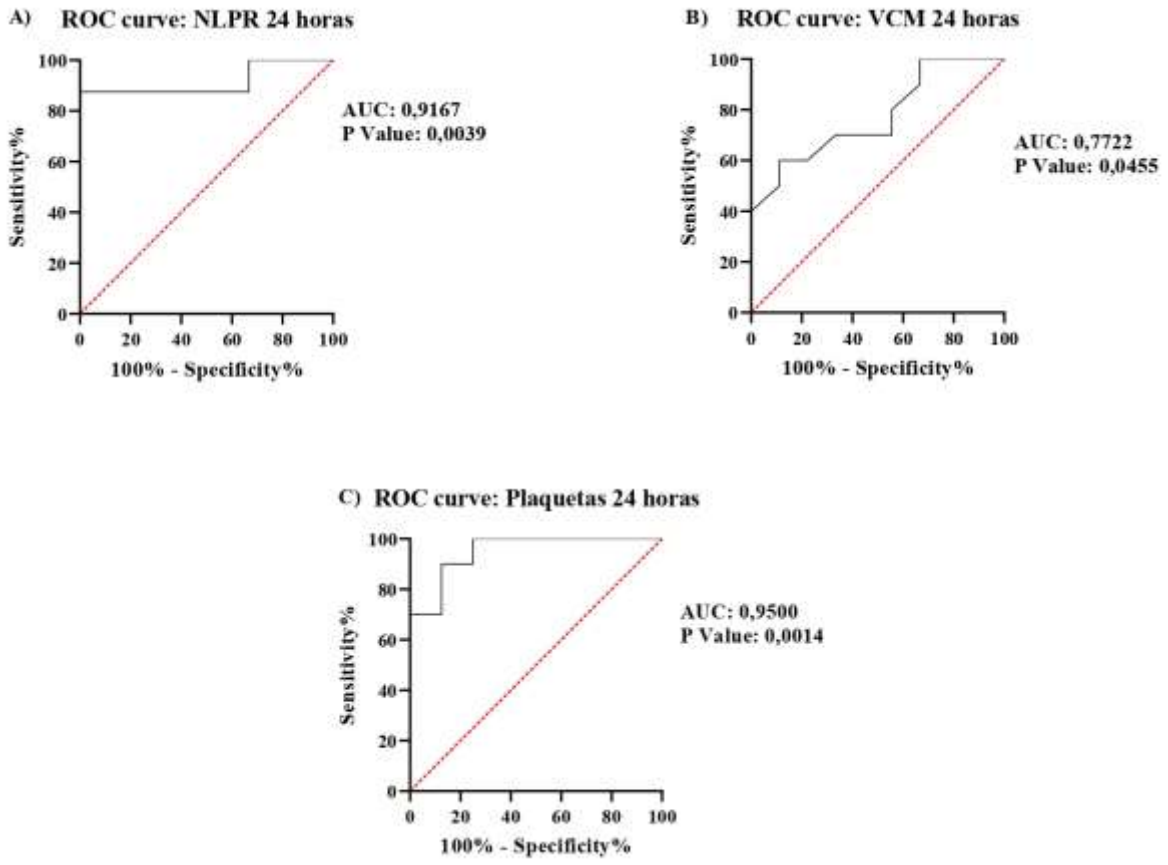


Legend: AUC: Area under the ROC curve.
Source: Prepared by the authors.

In the context of the study, the area under the curve (AUC) for glycemia, shown in Figure 4A, was identified as a highly promising indicator, with a result of 0.8958. A cutoff point of <123.5 mg/dL was established, with 90% sensitivity and 83.33% specificity. These results suggest that glycemia may serve as an effective tool for the early identification of patients at risk of developing sepsis, especially after 4 hours. It is essential to highlight that, for a more accurate assessment of sepsis risk, glycemia should be considered in combination with other clinical indicators.

Regarding Figure 4B, the AUC of 0.8889 shows that temperature may also serve as a relevant indicator for identifying patients at risk of sepsis, although its AUC is slightly lower than that of glycemia. The cutoff point of <35.95 °C demonstrated a high specificity of 100%, meaning that, when temperature is below this value, the likelihood of being at risk of sepsis is very high. However, the sensitivity of 77.78% indicates that a proportion of patients at risk of sepsis may not be identified solely based on temperature.

Figure 5 – ROC curve 24 hours after induction, NLPR, MCV, and platelets.



Legend: AUC: Area under the ROC curve. NLPR: Neutrophil-to-Lymphocyte and Platelet Ratio. MCV: Mean Corpuscular Volume.
Source: Prepared by the authors.

In Figure 5A, the AUC of 0.9167 for NLPR demonstrates a promising relationship at the 24-hour time point. The established cutoff point of >0.03488 mm³ showed a sensitivity of 87.50% and a notable specificity of 100%. These results support the use of NLPR as a valuable tool for the early detection of sepsis, thereby minimizing diagnostic errors. This suggests that this ratio may serve as a robust indicator for identifying patients at risk of developing sepsis.

Our results in Figure 5B indicate that the AUC of 0.7722 for MCV at 24 hours after induction demonstrates relevance, although with slightly lower sensitivity compared to NLPR. The cutoff point of <43.65 μ^3 revealed a sensitivity of 60% and a specificity of 77.78%. These results indicate that, while MCV is effective, its sensitivity and specificity should be interpreted along with other clinical markers to enhance diagnostic accuracy.

For platelet values at the 24-hour time point, as shown in Figure 5C, with a remarkable AUC of 0.9500, their potential in identifying sepsis becomes evident. The cutoff point of <954 thousand/mm³, aligned with reference values for mice, demonstrated a sensitivity of 90% and a specificity of 87.50%. This shows that platelet counts have a strong predictive capacity for sepsis-related outcomes, maintaining high sensitivity and specificity relative to mouse reference values.

When platelet counts are considered together with NLPR at 24 hours after induction, excellent sensitivity and specificity are revealed, indicating that combining these clinical indicators may be beneficial for improving accuracy in identifying sepsis risk. Overall, the results strengthen the rationale for using available clinical tools in the early detection of this critical condition.

CONCLUSION

Hypothermia and decreased glycemia may serve as important early and sensitive indicators of sepsis severity and assist in the diagnosis of the disease. These variables should be correlated with hematological parameters, such as MCV, platelet count, and the NLPR index, in order to be used both in monitoring sepsis and in predicting the worsening of the condition. Thus, this study demonstrates that hypothermia, glycemia, and hematological parameters may serve as important biomarkers for both the diagnosis and monitoring of sepsis-related complications.

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Submitted: December 28, 2023

Accepted: March 3, 2026

Published: March 25, 2026

GLYCEMIA, TEMPERATURE, AND HEMATOLOGICAL VARIABLES AS BIOMARKERS IN A MOUSE MODEL OF EXPERIMENTAL SEPSIS

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All authors approved the final version of the manuscript.	
Conflict of Interest:	There is no conflict of interest.
Funding:	This research received no external funding.
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GLYCEMIA, TEMPERATURE, AND HEMATOLOGICAL VARIABLES AS BIOMARKERS IN A MOUSE MODEL OF EXPERIMENTAL SEPSIS

APPENDIX A – CEUA APPROVAL CERTIFICATE.

UNIJUÍ UNIVERSIDADE REGIONAL | CEUA Comissão de Ética no Uso de Animais da UNIJUÍ

CERTIFICADO DE APROVAÇÃO

Comissão de Ética no Uso de Animais da UNIJUÍ

Certificamos que a proposta intitulada "**Efeito Farmacogenômico do Desbalanço de Superóxido no Tratamento da Sepse com HSPS**" registrada com o nº de protocolo 008/21, sob a responsabilidade de **Thiago Gomes Heck**, da Universidade Regional do Noroeste do Estado do Rio Grande do Sul, - que envolve a produção, manutenção ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto humanos), para fins de pesquisa científica (ou ensino) - encontra-se de acordo com os preceitos da Lei nº 11.794, de 8 de outubro de 2008, do Decreto nº 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle de Experimentação Animal (CONCEA), e foi aprovada pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS - CEUA/UNIJUÍ, em reunião no dia 26/04/2021.

Finalidade: Pesquisa	Espécie animal: Camundongo Isogênico
Data de protocolo:19/04/2021	Quantidade: 98
Vigência da autorização: 10/06/2021 a 31/12/2023	Sexo: machos
Origem do animal: Biotério Unijuí	Idade e peso: variável

Profª Drª Lucisna Mori Viero
Coordenadora da CEUA/UNIJUÍ

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GLYCEMIA, TEMPERATURE, AND HEMATOLOGICAL VARIABLES AS BIOMARKERS IN A MOUSE MODEL OF EXPERIMENTAL SEPSIS

APPENDIX B - Supplementary Table – Clinical and laboratory parameters expressed as measures of central tendency (mean), dispersion (standard deviation), and P-value for the period from 0 to 24 hours of the experiment.

PARAMETERS	0 HOURS		P Value	04 HOURS		P Value	12 HOURS		P Value	24 HOURS		P Value
	CONTROL	SEPSIS		CONTROL	SEPSIS		CONTROL	SEPSIS		CONTROL	SEPSIS	
	Mean and Standard Deviation			Mean and Standard Deviation			Mean and Standard Deviation			Mean and Standard Deviation		
RBC ($x10^6/mm^3$)	7.115 ± 2.663	7.928 ± 3.302	0.5047	7.295 ± 3.698	7.927 ± 2.468	0.5849	6.766 ± 2.484	8.037± 3.359	0.2848	6.307 ± 1.345	7.013 ± 2.328	0.4419
HGB (g/dL)	10.25 ± 3.671	11.06 ± 2.617	0.519	12.05 ± 4.883	12.68 ± 4.365	0.224	11.18 ± 3.150	12.19 ± 4.638	0.5286	9.767 ± 1.538	10.53 ± 3.043	0.5095
HCT (%)	31.66 ± 11.72	32.84 ± 7.817	0.7611	33.05 ± 18.30	34.92 ± 10.99	0.7335	31.64 ± 10.77	36.55 ± 15.69	0.6485	28.50 ± 6.203	30.70 ± 10.78	0.6029
MCV (μ^3)	44.68 ± 1.415	44.19 ± 2.116	0.5137	44.62 ± 3.479	44.32 ± 2.400	0.7884	44.15 ± 3.900	44.06 ± 2.667	0.2913	45.18 ± 1.762	43.59 ± 1.261	**0.0266***
RDW (%)	16.61 ± 1.797	16.33 ± 1.704	0.6807	16.65 ± 1.833	17.05 ± 2.509	0.6543	15.89 ± 3.269	16.33 ± 2.375	0.6712	16.18 ± 1.325	15.96 ± 1.482	0.7417
MCH (pg)	14.85 ± 3.334	14.84 ± 1.131	0.9953	17.56 ± 3.443	15.74 ± 3.462	0.3339	15.80 ± 1.851	15.45 ± 1.799	0.4216	15.70 ± 1.465	14.56 ± 0.6844	0.0554
MCHC (%)	33,19 ± 7.272	33.80 ± 2.922	0.7704	39.55 ± 8.215	37.31 ± 4.654	0.3643	33.94 ± 2.391	33.47 ± 1.816	0.8365	34.78 ± 3.679	33.35 ± 2.084	0.3492
WBC (mm^3)	3000 ± 1077	2725 ± 1169	0.5755	3810 ± 1631	3442 ± 1659	0.5729	4175 ± 3042	2850 ± 1375	0.1007	4567 ± 1817	3060 ± 1826	0.0897
#NEUT (mm^3)	407.7 ± 219.3	531.4 ± 408.6	0.9898	773.7 ± 632.2	464.4 ± 444.6	0.1764	509.0 ± 317.8	531.5 ± 411.6	0.5482	793.3 ± 544.1	727.0 ± 443.4	0.7804
#MON (mm^3)	34.91 ± 49.55	34.29 ± 44.24	0.9661	62.18 ± 64.39	26.00 ± 36.96	0.1322	31.20 ± 52.75	126.5 ± 151.1	0.1597	112.3 ± 117.7	19.00 ± 22.26	0.0802
#LINF (mm^3)	2595 ± 1055	2355 ± 922.2	0.5675	3038 ± 1352	2625 ± 1403	0.4719	3535 ± 2726	2275 ± 1084	0.223	3612 ± 1352	2327 ± 1486	0.073
LNR (mm^3/ mm^3)	0.151 ± 0.081	0.155 ± 0.070	0.8929	0.176 ± 0.1070	0.179 ± 0.119	0.9433	0.172 ± 0.076	0.231 ± 0.150	0.218	0.203 ± 0.109	0.377 ± 0.266	0.1135
LMR (mm^3/ mm^3)	0.020 ± 0.013	0.027 ± 0.007	0.3091	0.03 ± 0.030	0.055 ± 0.036	0.3437	0.048 ± 0.041	0.074 ± 0.049	0.3391	0.030 ± 0.030	0.033 ± 0.037	0.7811
LPR (mm^3/ mm^3)	0.463 ± 0.282	0.506 ± 0.259	0.6867	0.422 ± 0.248	0.634 ± 0.370	0.1166	0.547 ± 0.407	0.548 ± 0.302	0.9947	0.300 ± 0.092	0.519 ± 0.399	0.1285
NLPR (mm^3/ mm^3/ mm^3)	0.014 ± 0.007	0.018 ± 0.013	0.7675	0.019 ± 0.012	0.016 ± 0.012	0.7348	0.014 ± 0.007	0.028 ± 0.023	0.1912	0.020 ± 0.010	0.040 ± 0.013	**0.0028***
SII (mm^3/ mm^3/ mm^3)	164.2 ± 102.5	169.5 ± 89.09	0.8201	198.6 ± 125.4	259.6 ± 213.5	0.7352	205 ± 125.7	191.2 ± 97.16	0.7458	211.8 ± 119.4	290.0 ± 213.7	0.3521
AISI (mm^3/ mm^3/ mm^3/ mm^3)	8084 ± 3765	7996 ± 3591	0.9693	11518 ± 8125	38359 ± 35704	0.0935	23034 ± 28474	38330 ± 34617	0.2109	31081 ± 25805	25564 ± 28395	0.7103
SIRI (mm^3/ mm^3/ mm^3)	8.094 ± 4.670	9.665 ± 6.152	>0,9999	10.78 ± 6.586	36.70 ± 39.27	0.1215	18.62 ± 20.66	50.06 ± 55.73	0.1791	28.25 ± 23.05	34.71 ± 37.72	0.7058
PLT ($x10^3/mm^3$)	1083 ± 180.1	1145 ± 404.1	0.6379	1178 ± 401.7	1535 ± 733.2	0.158	1186 ± 346.0	966.9 ± 408.6	0.1356	1077 ± 97.48	763.8 ± 190.3	**0.0007***