

Adapted Murine Sepsis Score: Improving The Research In Experimental Sepsis Mice Model

Maicon Machado Sulzbacher

Universidade Federal de Santa Maria

Lucas Machado Sulzbacher

Universidade Regional do Noroeste do Estado do Rio Grande do Sul

Felipe Rafael Passos

Universidade Regional do Noroeste do Estado do Rio Grande do Sul

Bruna Letícia Endl Bilibio

Universidade Regional do Noroeste do Estado do Rio Grande do Sul

Kauana de Oliveira

Universidade Regional do Noroeste do Estado do Rio Grande do Sul

Wellington Felipe Althaus

Universidade Regional do Noroeste do Estado do Rio Grande do Sul

Matias Nunes Frizzo

Universidade Regional do Noroeste do Estado do Rio Grande do Sul

Mirna Stela Ludwig

Universidade Regional do Noroeste do Estado do Rio Grande do Sul

Ivana Beatrice Mânica Da Cruz

Universidade Federal de Santa Maria

Thiago Gomes Heck (✉ thiago.heck@unijui.edu.br)

Regional University of Northwestern Rio Grande do Sul State (UNIJUI) <https://orcid.org/0000-0002-1242-5423>

Research note

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Abstract

Objective

The Murine Sepsis Score (MSS) is used to assess the severity of sepsis in rats and mice based on observational characteristics. The quantitative variables of blood glucose, body weight and temperature are predictors of severity in experimental models of sepsis. Therefore, our study sought to adapt the MSS with the same variables to indicate earlier the severity of the disease in murine models of the disease.

Results

Sepsis mice presented hypoglycemia, weight loss, and hypothermia. Therefore, these variables were included in the Adapted Murine Sepsis Score (A-MSS). The A-MASS presented 100% specificity and 87.5% sensibility been able to differentiate the early sepsis symptoms and its severity. The A-MSS allows an early and more complete diagnosis of sepsis in mice and might be considered as a procedure to improve the analysis of systemic sepsis dysfunction in murine experimental models.

Introduction

Sepsis is a systemic infection characterized by multiple organ dysfunction and high mortality rates. Therefore, studies investigating the pathophysiology in sepsis are required [1, 2], and the use of experimental mouse models has been considered a fundamental approach [3]. The bacteria-induced sepsis in animal models has similar characteristics found in septic patients [4, 5] due to the bacteria membrane endotoxins like lipopolysaccharide (LPS) and lipoteichoic acid (LTA) [6].

One of the leading infectious focus in sepsis is abdominal origin. *Staphylococcus aureus* and *Escherichia coli* bacteria are commensals of the mouse gastrointestinal tract and can be found in the feces of these animals [5, 7]. Therefore, an experimental model of sepsis is peritonitis induction by the intraperitoneal administration of the autogenous fecal solution. From this procedure, sepsis is recognized by an application of Murine Sepsis Score (MSS) that consists of observing and categorizing the level of consciousness, activity, behavior, response to stimuli, respiratory rate, and quality of breathing movements [8]. However, it is possible to observe a decreased glycemic levels, core temperature, and body weight loss if we follow up animals for 24 hours [9, 10], but these variables have not been included in the MSS protocol [8].

Patients and animals with sepsis may present hypothermia [11, 12], hypoglycemia [9, 13], body weight loss [14], and hematological alterations [15]. Also, early identification of sepsis is a well-known, relevant procedure to allow successful treatment of the disease [16]. Therefore, herein we proposed an adaptation in the MSS (A-MSS) to provide a score that identifies sepsis in an experimental model in the initial stages.

Methods

Animals

Fifteen male C57BL/6 mice aged 90 to 150 days from the Life Sciences Department (DCVida) of the Northwestern Regional University of Rio Grande do Sul State (UNIJUÍ) were used in this study.

Experimental design

The animals were randomly divided into two experimental groups: Control (n = 7) and Sepsis (n = 8). A 20% fecal solution (200 mg/mL) was prepared with fresh stool in 0.9% NaCl, and it was administered at a dose of 5 μ L/g (1 mg/g, i.p.) in sepsis group, while Control animals received 0.9% NaCl [10]. The animals were monitored for blood glucose, blood count, rectal temperature and MSS (Table S1), before (time zero) and at 4, 12, and 24 hours after sepsis induction and were euthanized by decapitation using guillotine. Sample size was defined considering the sufficient number to find difference between groups in MSS [10].

Procedures details

Glycemia was measured by distal tail puncture (~5 μ L) using Optium Xceed® glucometer. Rectal temperature was measured with a digital thermometer, and body weight was measured using a semi-analytical balance. For hematological analysis, blood was collected (0, 4, 12 and 24 h) by caudal puncture (10 μ l). Samples were diluted 1:3 with 0.9% saline, 1 μ l of anticoagulant (EDTA). Hematological strains were performed on the slide for differential leukocyte count, stained with a panoptic type [21].

Statistical analysis

Data were analyzed using the Kolmogorov-Smirnov test. The results in which the evaluation was performed over time were verified by two-way ANOVA (time x treatment) followed by Bonferroni post-test. Spearman or Pearson correlation test was performed. The potential diagnosis of A-MSS within four hours after sepsis induction was tested by ROC curve. The cutoff point for A-MSS diagnosis was determined by observing the most appropriate balance between sensitivity and specificity in the ROC curve [22]. The significance level of 5% (P <0.05) was considered.

Results

The animals submitted to sepsis presented increased MSS 12 h and 24 h after fecal solution administration (Fig. 1a). Also, animals with sepsis showed a decrease in glycemia, rectal temperature and body weight (Fig. 1b, c, d).

It was possible to verify that sepsis induces alterations in appearance, level of consciousness, activity, stimulus-response, eye aspect and respiratory rate and quality (Figure S1).

Sepsis was able to cause a decrease in absolute and relative platelet count (Figure S2a,b), and increases mean platelet volume (MPV), and the ratio between MPV and platelet count (MPV/PC) (Figure S2c,d). The neutrophil count increased after 12 h, returning to basal levels 24 h after sepsis induction (Figure S3a). At 24 h we found a decrease in leukocyte count, which includes a decrease in lymphocyte and monocyte count (Figure S3b,c,d, respectively). These alterations in immune cells, as well as observed in the platelets, impact in the neutrophil-lymphocyte ratio (NLR), which increased at 12 h together with the platelet to lymphocyte ratio (PLR) in 24 h (Figure S3e,f, respectively).

A negative correlation was observed between MSS and glycemia, body temperature and weight loss, 24 hours after sepsis induction (Fig. 2a-c). Therefore, we proposed the A-MSS (Table 1).

The A-MSS was able to report sepsis within four hours of the sepsis induction (Fig. 2d). When this score was evaluated from the ROC curve in this period, it was found that it can be used as a diagnostic standard (Fig. 2e), from an A-MSS value of 3.5, obtaining a sensitivity of 87.5% and specificity of 100%.

A-MSS measured four hours after sepsis induction showed a negative correlation with the severity marker parameters in experimental sepsis: decrease in lymphocyte count, total leukocytes and platelets (Figure S4a-c). Also, there was a correlation with the increase in NLR, PLR and MPV/PC (Figure S4d-f).

Discussion

We showed that the inclusion of variables easily measurables as glycemia, temperature, and body weight in the MSS may improve the research in sepsis mice model. Our proposal of A-MSS represents a sum of observations, and together with the established MSS, the above-mentioned variables might be considered as a new score for the evaluation of sepsis in experimental models (Table 1).

Table 1
Adapted Murine Sepsis Score (A-MSS)

Score	0	1	2	3	4
Appearance	Coat is smooth	Patches of hair piloerected	Majority of back is piloerected	Piloerection may or may not be present, mouse appears "puffy"	Piloerection may or may not be present, mouse appears emaciated
Level of consciousness	Mouse is active	Mouse is active but avoids standing upright	Mouse activity is noticeably slowed. The mouse is still ambulant	Activity is impaired. Mouse only moves when provoked, movements have a tremor	Activity severely impaired. Remains stationary when provoked, with possible tremor
Activity	Normal amount of activity. Mouse is any of: eating, drinking, climbing, running, fighting	Slightly suppressed activity. Mouse is moving around bottom of cage	Suppressed activity. Mouse is stationary with occasional investigative movements	No activity	No activity. Mouse experiencing tremors, particularly in the hind legs
Response to stimulus	Mouse responds immediately to auditory stimulus or touch	Slow or no response to auditory stimulus; strong response to touch (moves to escape)	No response to auditory stimulus; moderate response to touch (moves a few steps)	No response to auditory stimulus; mild response to touch (no locomotion)	No response to auditory stimulus. Little or no response to touch. Cannot right itself if pushed over
Eyes	Open	Eyes not fully open, possibly with secretions	Eyes at least half closed, possibly with secretions	Eyes half closed or more, possibly with secretions	Eyes closed or milky
Respiration rate	Normal, rapid mouse respiration	Slightly decreased respiration (rate not quantifiable by eye)	Moderately reduced respiration (rate at the upper range of quantifying by eye)	Severely reduced respiration (rate easily countable by eye, 0.5 s between breaths)	Extremely reduced respiration (> 1 s between breaths)

Score	0	1	2	3	4
Respiration quality	Normal	Brief periods of laboured breathing	Laboured, no gasping	Laboured with intermittent gasps	Gasping
Rectal temperature (°C)	36–38	> 38	< 36 - ≥ 35	< 35 - ≥ 34	< 34
Casual blood glucose (mg/dL)	≥148	≤ 148 - ≥122	< 122 - ≥58	<58 - >40	≤ 40
Relative body weight loss (%)	0–5	5–10	10–15	15–20	> 20

Sepsis is a complex disease that requires a complex form to diagnose it successfully. [25]. The A-MSS added parameters to the MSS, which allowed an indirect assessment of the cardiovascular (temperature) and metabolic systems (glycemia and body weight), which are essential for the prognosis in sepsis in animals and humans. In humans, the diagnosis is made by measures of neurological, cardiovascular, respiratory, renal, hepatic, and platelet dysfunction, by the reproducible Sequential Organ Failure Assessment (SOFA) score. [26]. We believe that A-MSS approximates the clinical variables evaluated in SOFA for the early diagnosis of sepsis in animal models.

The central nervous system dysfunction is mainly characterized by septic encephalopathy, followed by autonomic failure [27] [27]. These dysfunctions cause tissue damage and impair brain function [28]. In our study, these neurological effects were reflected in the impairment of the level of consciousness and activity in the Sepsis group (Figure S1a,c). The neurological symptoms can be identified in the first hours by MSS [11], but using the A-MSS allows the assessment of autonomic insufficiency evaluating the temperature (Fig. 1c).

Neurohypophysial dysfunction promotes decreased hepatic gluconeogenesis-promoting adrenocortical hormones and liver and muscle glycogenolysis, causing a decrease in glycemia [29]. Furthermore, LPS is able to decrease the activity of hepatic and renal enzyme phosphoenolpyruvate carboxykinase (PEPCK) promoting gluconeogenesis, and hypoglycemia [30], as observed in Fig. 1b.

Sepsis impairs neuroendocrine regulation by neuronal dysfunction impairing the secretion of vasopressor hormones [31], accompanied by the desensitization of receptors for vasoconstriction [32]. Also, LPS and cytokines induce the nitric oxide production by macrophages, neutrophils and monocytes [32]. Decreased cardiac output and blood pressure may be associated with hypothermia (Fig. 1c) [11, 37], reinforcing the need to measure body temperature in sepsis mice models.

A catabolic condition leads to a reduction in muscle mass [38] is related to increased hospitalization period and mortality [14]. This metabolic effect is also verified in an experimental sepsis model [9, 10, 39],

and the results found in our study (Fig. 1d) may be associated with muscle atrophy and lipid catabolism [40]. Also, leukocytes release interleukin-1 beta, which has a direct effect on appetite inhibition and food intake, reflecting in body weight loss [41].

The platelet function disorders play a crucial role in the pathophysiology of sepsis, with prognostic accuracy [42]. LPS and cytokine endotoxemia (TNF- α , tumor necrosis factor-alpha, IL-8, IL-15) stimulate endothelium, monocytes, neutrophils and basophils to secrete platelet-activating factor (PAF) [42]. PAF triggers platelet aggregation with the formation of microthrombi, which, together with leukocyte-induced hemophagocytosis [43], may result in decreased total platelet count (thrombocytopenia) and its relative rate [44]. Regarding MPV, it is already known that the 24 h period is insufficient for the increase in MPV to indicate the severity in patients with sepsis [45], as observed in our study (Figure S2c). It has been proposed that MPV indicates severity in just after 72 hours [46] and that the evaluation of MPV is insufficient to predict the worsening of sepsis due to peritonitis with gram-negative bacteria [15]. The dysfunction in the platelet count can also be observed by analyzing the ratios between the MPV and platelet count (MPV/CP), representing the risk of microthrombi formation [47]. The platelet is a predictor of worsening of sepsis, specifically when it accuses the systemic infection with gram-negative bacteria [15], as we observed in our study (Figure S2d).

Immunosuppression observed by decreasing lymphocyte and monocytes leads to a decrease in total leukocytes (Figure S3b,c,d). On the other hand, the bone marrow has a reservoir of neutrophils that are released into the circulation to combat infectious in the peritoneal cavity, as verified 12 h after sepsis induction [48]. Neutrophils are immune cells of the first line of defense against bacterial infection and may suffer exacerbated apoptosis in severe sepsis [15].

Studies suggest the application of NLR and PLR as inflammatory biomarkers [15]. PLR and NLR elevation is a prognostic marker of lethality in patients with peritonitis [15], as well as in an animal models [49], similarly to Figure S3e,f. Also, platelet and immune biomarkers may indicate etiological agents [15]. Thus, the A-MSS proposed in our study was able to indicate sepsis in mice just after four hours, correlated with the 24-hour values of the biomarkers mentioned above (Figure S3a-f).

Conclusion

The A-MSS allows an early and more complete diagnosis of sepsis in mice and might be considered as a procedure to improve the analysis of systemic sepsis dysfunction in mice.

Limitations

Our study did not use laboratory tests for sepsis evaluations such as bilirubin, creatinine or lactate.

Abbreviations

LPS: lipopolysaccharide, LTA:lipoteichoic acid, MSS:Murine Sepsis Score, A-MSS:Adapted Murine Sepsis Score, NaCl:sodium chloride, ROC:Receiver Operating Characteristic, MPV:mean platelet volume, MPV/PC:ratio between MPV and platelet count, NLR:neutrophil-lymphocyte ratio, PLR:platelet to lymphocyte ratio, M-CASS:Mouse Clinical Assessment Score for Sepsis, SOFA:Sequential Organ Failure Assessment, CNS:central nervous system, PEPCK:phosphoenolpyruvate carboxykinase, iNOS:inducible nitric oxide synthase, NO:nitric oxide, IL-1 β :interleukin-1 beta, TNF- α :tumor necrosis factor alpha, PAF:platelet-activating factor.

Declarations

Availability of data and materials

All data will be available under request for authors.

Ethics approval

The ethical principles established by the International Animal Protection Standards have been respected [17], Brazilian Code of Animal Experimentation - 1988, as well as the National Institutes of Health (NIH) Guide to Laboratory Animal Care and Use. This study was approved by the Animal Use Ethics Committee of UNIJUÍ (CEUA 048/2016).

Consent for publication

All authors approved the submitted and published versions.

Competing interests

All authors declared have no conflict of interest.

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Authors' contributions

M.M.S. performed all of the experiments described in this manuscript with laboratory colleagues. M.M.S. performed the administration of fecal solution in the mice. The murine sepsis score was evaluated by M.M.S.. B.L.E.B., M.M.S., L.M.S. and K.O. performed the biometric and metabolic profile procedures.

M.M.S. and L.M.S. performed the western blot analyses. M.N.F., W.F.A. and M.M.S. performed the hematological procedures. All authors were involved in analyzing the results. T.G.H., I.B.M.C. and M.M.S. performed statistical analyses and co-wrote the manuscript. T.G.H., M.S.L. and M.M.S. designed the study, provided experimental advice, and helped with manuscript revision.

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Figures

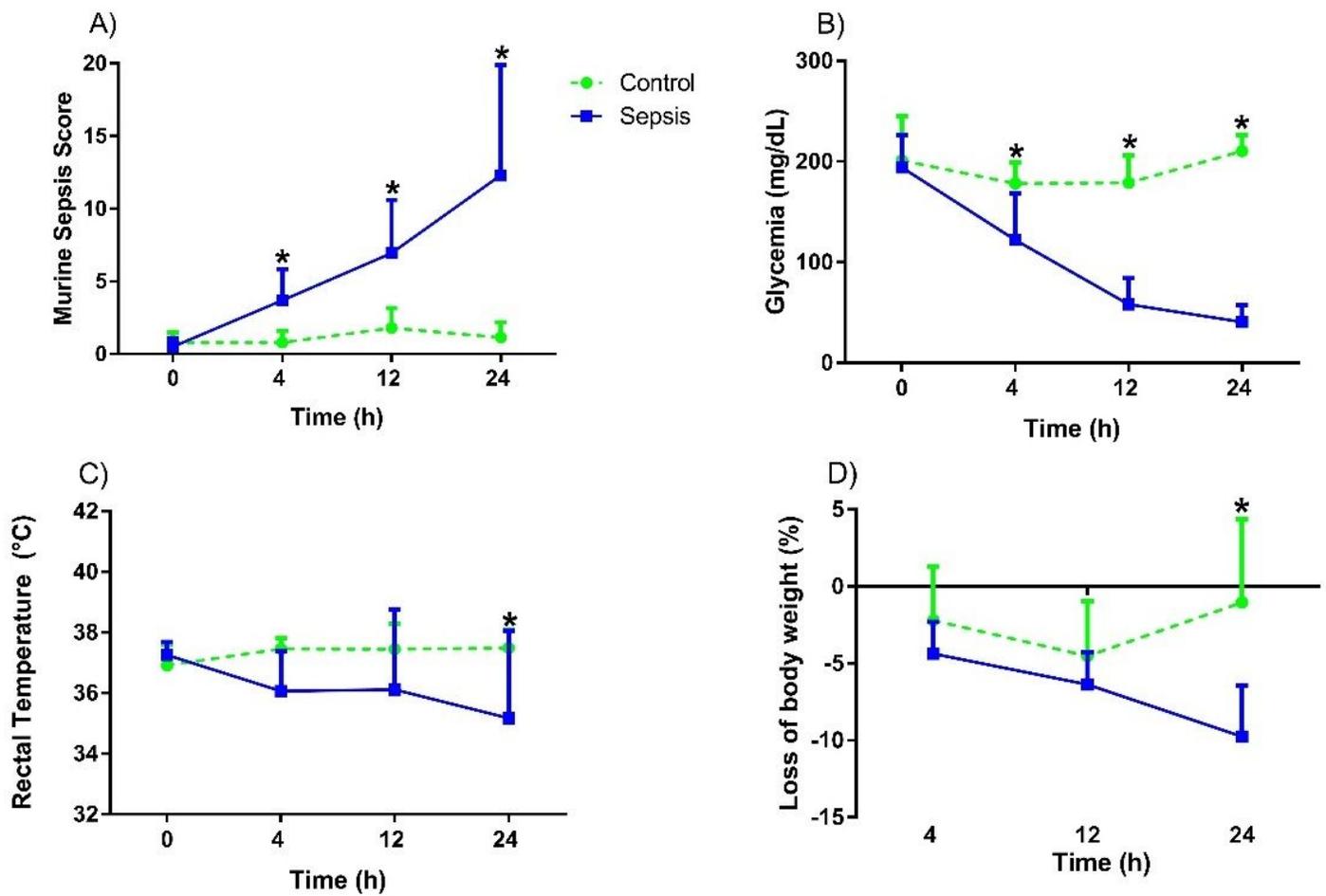


Figure 1

Characterization of sepsis model in mice. a Measurement of murine sepsis score (MSS) (* $P < 0,0001$; $F_{1,52} 31,43$); is Blood glucose (* $P < 0,0001$; $F_{1,52} 124,3$); c Rectal temperature (* $P < 0,0001$; $F_{1,130} = 8,23$); d Body weight loss (* $P < 0,0002$; $F_{1,39} - 16,95$) within 24 hours after induction of sepsis with 20% faecal solution (1 mg/g).

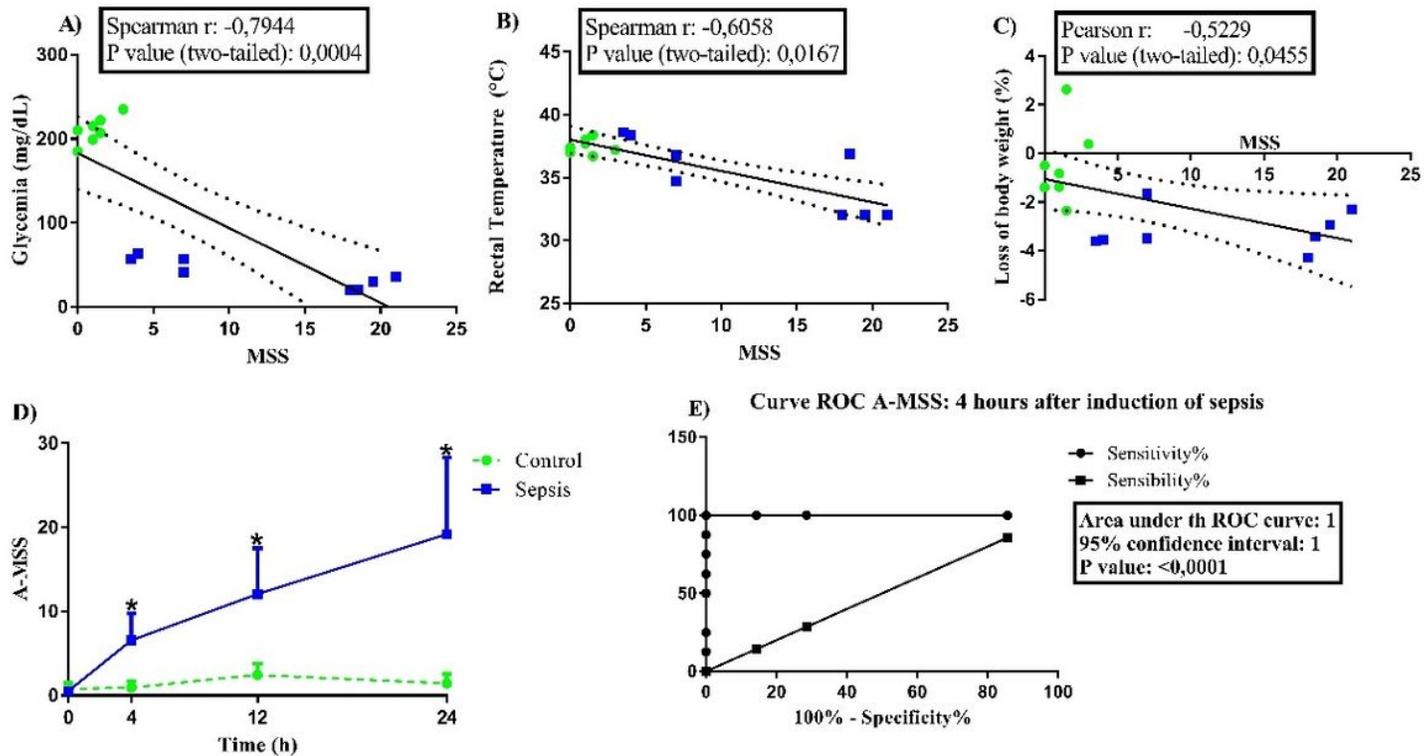


Figure 2

Correlations between MSS and measured parameters and the elaboration of A-MSS. a Correlation between MSS and blood glucose, b temperature and c relative weight loss in 24 hours after sepsis induction, and measurement of EI A-MSS throughout the experiment period (* P, 0,0001; P1,52 = 58,39) (D) and e ROC curve at tint 4 hours after induction of sepsis with 20% faecal solution (l mg/g).

Supplementary Files

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