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ORIGINAL ARTICLE

Association between severity score, inflammatory levels and DNA damage in intensive care patients

Associação entre escore de gravidade, níveis inflamatórios e dano no DNA em pacientes na terapia intensiva

Asociación entre puntaje de gravedad, niveles inflamatorios y daño en el ADN en pacientes de cuidados intensivos

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ABSTRACT

Background and Objectives: The Intensive Care Unit (ICU) is responsible for the treatment of critical patients and monitoring it continuously can improve the quality of care provided. This study aims to associate the Simplified Acute Physiology Score (SAPS 3) with inflammatory levels and genomic damage in patients admitted to the ICU of a hospital in Vale do Taquari, Rio Grande do Sul, Brazil. **Methods:** This is a cross-sectional study conducted with 22 patients from an adult ICU, between January and June 2016. The SAPS 3 was scored by the medical staff at the admission of patients and blood samples were obtained after 24 and 72 hours of hospitalization for C-Reactive Protein (CRP) dosing and DNA damage. **Results:** The SAPS 3 score was not associated with 24- and 72-hours CRP. However, the SAPS 3 score was significantly associated with the index and frequency of DNA damage, only after 72 hours of hospitalization. **Conclusion:** The severity score was not associated with CRP levels, but with DNA damage only after 72 hours of admission.

Keywords: *Critical Care. Simplified Acute Physiology Score. Polymerase Chain Reaction. DNA damage.*

RESUMO

Justificativa e Objetivos: A Unidade de Terapia Intensiva (UTI) é responsável pelo tratamento de pacientes críticos e sua monitorização contínua pode melhorar a qualidade dos cuidados prestados. O objetivo deste estudo é associar a Escala Psicológica Aguda Simplificada (SAPS 3) com os níveis inflamatórios e o dano ao DNA em pacientes

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internados na UTI de um hospital do Vale do Taquari, Rio Grande do Sul, Brasil. **Métodos:** Trata-se de uma pesquisa transversal realizada com 22 pacientes internados em uma UTI adulta, no período de janeiro a junho de 2016. O escore SAPS 3 foi pontuado pela equipe médica na admissão dos pacientes e amostras sanguíneas foram obtidas após 24 e 72 horas de internação para dosagem de Proteína C Reativa (PCR) e dano no DNA. **Resultados:** O escore SAPS 3 não se associou ao PCR de 24 e 72h. Entretanto, o escore SAPS 3 associou-se significativamente ao índice e a frequência de dano DNA, somente após 72 horas de internação. **Conclusão:** O escore de gravidade não se associou aos níveis de PCR, mas a danos no DNA, somente após 72 horas da admissão.

Descritores: *Cuidados Críticos. Escala Psicológica Aguda Simplificada. PCR. Dano ao DNA.*

RESUMEN

Justificación y objetivos: La Unidad de Cuidados Intensivos (UCI) es responsable del tratamiento de pacientes críticos, y su monitoreo continuo puede mejorar la calidad de la atención ofrecida. El presente estudio tuvo como objetivo asociar la Puntuación Fisiológica Simplificada Aguda (SAPS 3) con los niveles inflamatorios y el daño al ADN en pacientes de la UCI de un hospital del Valle de Taquari, Rio Grande do Sul, Brasil. **Métodos:** Este es un estudio transversal realizado con 22 pacientes ingresados en una UCI de adultos, entre enero y junio de 2016. El equipo médico calificó la puntuación SAPS 3 al ingreso de los pacientes, y se obtuvieron muestras de sangre después de 24 y 72 h de hospitalización para la medición del PCR y el daño al ADN. **Resultados:** La puntuación SAPS 3 no se asoció con la Proteína C Reactiva (PCR) a 24 y 72 horas. Sin embargo, lo asoció significativamente con el índice y la frecuencia de daño al ADN solo después de 72 horas de hospitalización. **Conclusiones:** El puntaje de gravedad no se asoció con los niveles de PCR, sino con el daño al ADN solamente 72 horas después del ingreso de los pacientes.

Palabras clave: *Cuidados Críticos. Puntuación Fisiológica Simplificada Aguda. Reacción en Cadena de la Polimerasa. Daño del ADN.*

INTRODUCTION

The Intensive Care Unit (ICU) is the hospital environment for treating critically patients, who require continuous monitoring for stabilization and improvement of their clinical conditions.[1] The evaluation of this hospital unit's performance is necessary to develop and to improve routine strategies, aiming to enhance the quality of care provided for patients.[2]

The simplified acute physiology score (SAPS 3) is considered an important prognostic protocol in the ICU to identify the most severe patients who should receive systematized care.[3,4] Furthermore, C-reactive protein (CRP) represents an important supportive biomarker to determine the prognosis, expressed especially in the acute inflammatory phase. CRP has also been used as a prognostic and response biomarker for treatment in patients with ventilator-associated pneumonia (VAP), a very common infection in the ICU.[5,6]

DNA, which is responsible for the preservation and replacement of genetic information in cells, may also be damaged in association with some inflammatory processes. The comet assay, a versatile and adaptable method that have been applied for several years, can be used to determine the severity of these cell damages.[7] However, to date, there are no reports in the literature regarding the application of this trial in critically ill patients in the ICU.

Thus, this study aimed to associate the SAPS 3 score with inflammatory levels and DNA damage in ICU patients of a medium-sized hospital in the Taquari Valley, Rio Grande do Sul, Brazil.

METHODS

This study was evaluated and approved by the Research Ethics Committee of the Universidade de Santa Cruz do Sul (UNISC) (CAAE: 50606815.1.0000.5343) and by the Center for Studies and Research (Centro de Estudos e Pesquisa-CENEPE) of Hospital Bruno Born (HBB), Lajeado, Rio Grande do Sul, according to Resolution No. 466/12 of the Brazilian National Health Council (CNS).

This is a prospective observational, cross-sectional study with patients admitted to the adult ICU of the HBB between January and June 2016. All patients hospitalized less than 24 hours in the ICU of the hospital and who remained for at least 72 hours in the unit, who agreed to participate in the study, signing the informed consent form at the time of ICU stay, were included in the study. In the case of disabled patients, the permission was provided by their guardian. Blood samples from the participants were obtained during the first 24 hours of hospitalization and after 72 hours of hospitalization, for comet assay and CRP dosage. Patients who were transferred from other health institutions were excluded from the sample; those who were hospitalized in the institution for more than 48 hours in the last month; were diagnosed with pneumonia or maxillomandibular block (due to difficulty to sanitize); as well as those from whom it was not possible to collect at least two blood samples for laboratory tests were also excluded.

The variables of interest, referring to the participants, were collected and recorded in a control form including: age, sex, development of VAP (yes or no), reason for hospitalization, length of stay in the ICU, use

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of mechanical ventilation and antibiotics, SAPS 3 score, patient evolution, CRP values, and comet assay in the first and second collections (during the first 24 hours and after 72 hours of ICU stay, respectively). The verification carried out in the first 24 hours, evaluated the patient during ICU stay and after 72 hours, when one may develop VAP (pneumonia occurring after 48 hours, resulting from endotracheal intubation or tracheotomy to receive mechanical ventilation, as well as removal of the mechanical ventilation and extubation).[6]

The determination of the SAPS 3 score was performed at the patient's admission in the ICU to predict the prognosis of hospital mortality.[6] This measure is a routine practice of the unit, it is performed by the medical team workers in the first 24 hours after the patients' admission. The SAPS 3 score ranges between 0 and 217 points, and the higher the score identified, the higher the probability of patient's death.[8]

Blood samples (whole blood and serum), which were obtained from the laboratory of clinical analysis responsible for the routine examinations of hospitalized patients, were sent to the Experimental Nutrition Laboratory of UNISC for evaluation of DNA damage by comet assay and to the Exercise Biochemistry Laboratory of UNISC for CRP dosage.

The alkaline comet assay was performed according to the standard protocol of the Laboratory of Experimental Nutrition of UNISC.[9] A total of 100 cells per individual (50 per slide, two slides per individual), randomly selected, were analyzed under a conventional microscope with 200 times magnification. The damage was visually determined by cells classification (comet-shaped morphology) in five DNA migration classes, from damage 0 (no damage, circular morphology only "head" and no "tail") to damage 4 (maximum damage, "tail" bigger than the "head") (Figure 1). The damage index was obtained by the sum of individual cells classified, ranging between 0 (no damage: 100 cells times 0) and 400 (maximum damage: 100 cells times four). The frequency of damage, in percentage (%), was estimated by the relationship between the number of cells with damage (classified from 1-4) and the total cells of the sample. Non-detectable cells nuclei (NDCN) (separate head and tail) were not considered.

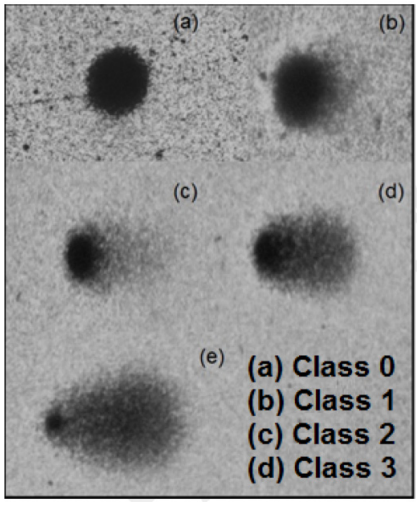

Figure 1. Typical images obtained with comet assay. Santa Cruz do Sul, RS, Brazil, 2016.

CPR was measured by immunoturbidimetry in the Miura 200 automated system (I.S.E., Rome, Italy), by DiaSys commercial kits (DiaSys Diagnostic Systems, Germany), at the Laboratory of Biochemistry and Exercise of UNISC. CRP values above 0.6 mg/dL were considered as indicative of inflammation and, the higher the value, the higher the intensity of inflammation.[10]

Data were analyzed in the Statiscal Package for Social Sciences (SPSS, IBM Corp, Somers, State) version 20.0 and the GraphPad Prisma version 6.01 program (Graphpad Inc., San Diego, USA) was used for chart plotting. Spearman's correlation was used to verify the relationship between CRP values, DNA damage, and SAPS score 3. A significance level of $p < 0.05$ was considered.

RESULTS

Between January and June 2016, 179 patients admitted to the adult ICU of HBB were considered eligible for the study, but only 64 blood samples were obtained. Out of these, 22 patients performed two blood collections to assess the level of DNA damage and CRP, on the day of ICU admission and 72 hours after hospitalization (Figure 2).

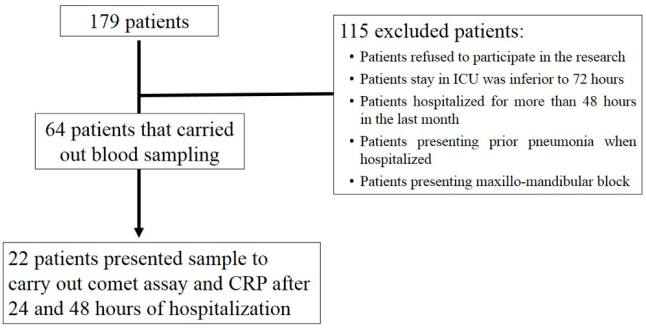

Figure 2. Flowchart of eligible participants who were admitted to the ICU of Hospital Bruno Born, Lajeado, RS, during the study and final sample number. Santa Cruz do Sul, RS, Brazil, 2016.

Table 1 shows the characteristics of the 22 patients included in this study. The mean age was 56.82 ± 18.88 years, most patients were men (81.8%) and they were admitted for surgical causes (72.7%). The mean length of hospital stay was about six days and the mortality rate was 13.6%. The average SAPS 3 score was close to 42 points.

CRP levels in the first 24 hours and after 72 hours of hospitalization were not associated with the SAPS 3 score presented by patients ($p > 0.05$) (Figure 3).

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Table 1. Characteristics of patients admitted to the ICU, participants of the sample (n=22). Santa Cruz do Sul, RS, Brazil, 2016.

Characteristic	Mean	±SD	N	%
Age (years)	56.82	18.88		
Sex				
Female			4	18.2
Male			18	81.8
Development of VAP/HAP				
Yes			3	13.6
No			19	86.4
Reason of hospitalization				
Clinic			6	27.3
Surgical			16	72.7
ICU stay (days)	5.64	3.57		
Mechanical Ventilation				
Yes			20	90.9
No			2	9.1
Use of antibiotic				
Yes			19	86.4
No			3	13.6
SAPS 3 score	41.64	14.00		
Patient evolution				
High			19	86.4
Death			3	13.6
Evaluation in the first 24 hours of hospitalization				
CRP (mg/dl)	6.65	5.45		
Damage index (u.a)	13.66	6.06		
Frequency of damage (%)	8.87	6.06		
Evaluation after 72 hours of hospitalization				
CRP (mg/dl)	4.14	3.06		
Damage index (u.a)	9.44	3.75		
Frequency of damage (%)	6.45	2.47		

SD: standard deviation; F: female; M: male; VAP: ventilator-associated pneumonia; HAP: hospital-acquired pneumonia; SAPS 3: simplified acute physiology score; CRP: C-reactive protein; ICU: intensive care unit.

There was no association between DNA damage index and SAPS 3 score in the first 24 hours of ICU stay ($p > 0.05$) (Figure 4: A and B). However, it was observed that the higher the severity score, the higher the index (r = 0.512; p = 0.015) (Figure 4: C) and the frequency of damage (r = 0.471; p = 0.027) (Figure 4: D) after 72 hours of hospitalization.

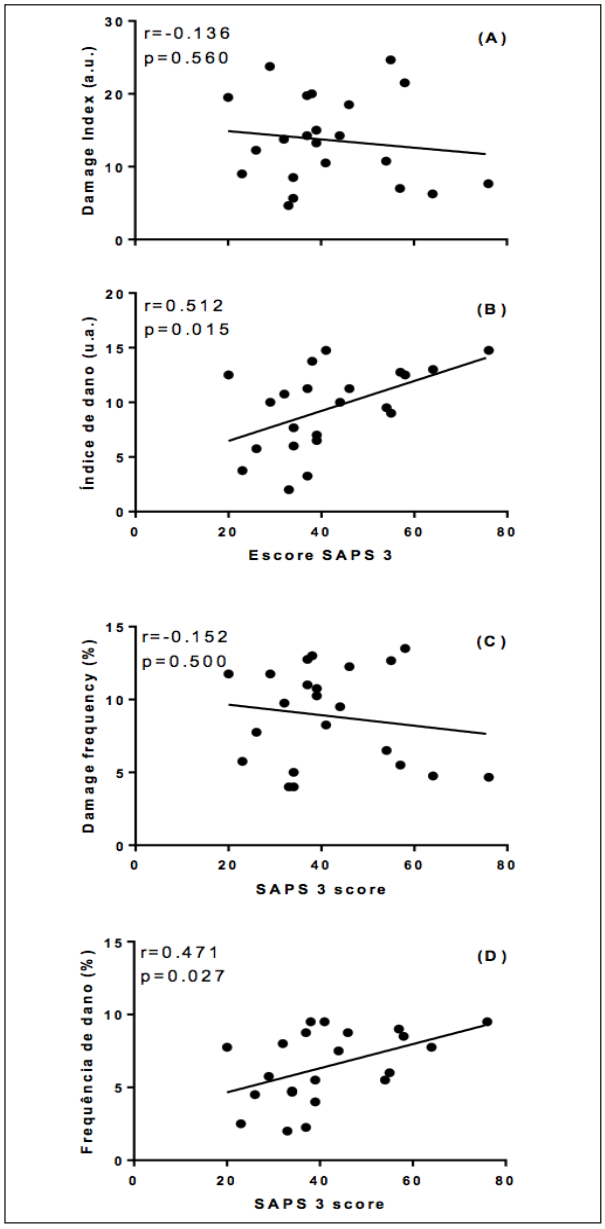

Figure 4. Relationship between DNA damage index (A, B) and frequency of DNA damage (C, D) with SAPS 3 score in the first 24 and 72 hours after ICU admission. r and p: correlation coefficient and significance level, respectively according to Spearman's correlation test. Santa Cruz do Sul, RS, Brazil, 2016.

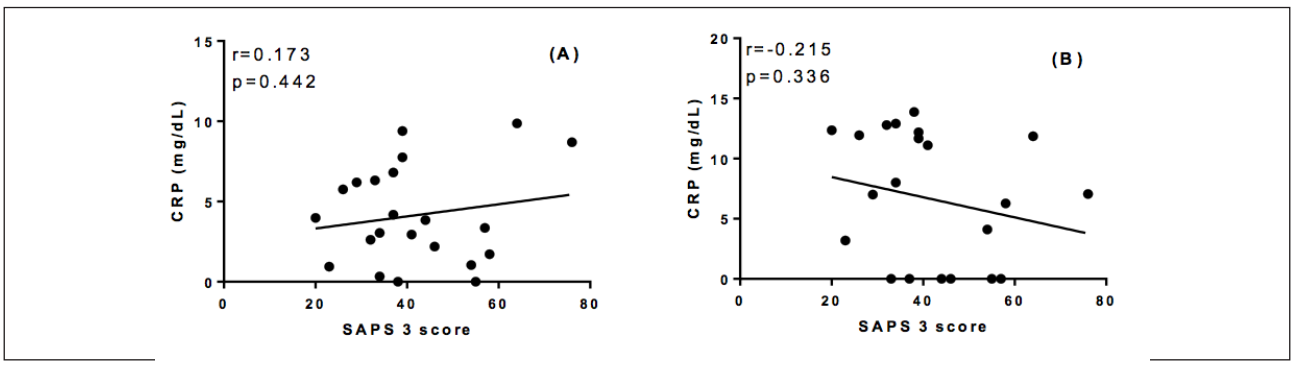

Figure 3. Relationship between CRP levels with SAPS 3 score in the first 24 hours (A) and 72 hours (B) after ICU admission. r and p: correlation coefficient and significance level, respectively, according to Spearman's correlation test. Santa Cruz do Sul, RS, Brazil, 2016.

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DISCUSSION

The SAPS 3 score was developed as a prognosis of hospital mortality at the patient's admission to the ICU, enabling the evaluation of the disease severity and the vital state at hospital discharge. In addition to evaluating the individual outcome of each patient, the index also evaluates the effectiveness of the practices used in the ICU.[3]

Based on the results found, it is worth mentioning that CRP levels were not associated with the severity score presented by the participating patients. One hypothesis for this finding would be that many patients are already admitted to the ICU with some level of inflammation or infection. Furthermore, it is known that CRP is an acute phase protein, that could also predict the prognosis or severity of bacterial infections, since it is highly sensitive to inflammatory processes. Therefore, serum CRP levels increase due to infectious causes as well as non-infectious causes of inflammation.[11]

Moreover, it has been evidenced that hospitalized patients present CRP levels greater than 5 mg/dL and the continuity of high levels or reduction inferior to half of the initial value indicates unfavorable evolution or complications.[12] A study conducted in Chinese ICUs reported that patients who developed VAP had significantly higher CRP levels than those who did not develop it.[13] In our study, only three of the 22 individuals evaluated developed VAP, making statistical analysis impossible.

Furthermore, it is known that critical disease is associated with oxidative stress, which can aggravate organ lesions and clinical evolution in general.[14,15] In this study, the severity score was significantly associated only with the index and the frequency of DNA damage after 72 hours of ICU stay. It is important to evaluate the patient after 72 hours, as considering that they may develop VAP in this period, pneumonia occurring after 48 hours, resulting from endotracheal intubation or tracheotomy to receive mechanical ventilation, as well as the removal of mechanical ventilation and extubation.[6]

However, a study evaluating 45 severely ill patients admitted to an adult ICU by SAPS II confirmed the presence of oxidative stress among participants.[16] Another study, evaluating oxidative stress in 139 critically ill patients with systemic inflammatory response syndrome, observed an increase in levels of thiobarbituric acid reactive substances(TBARS) in patients who developed multiple organ failure, i.e., the most severe.[17] These results can be compared to those of the present study, since the most severe patients had higher rates and frequencies of DNA damage.

This study showed that in critically ill patients admitted to the ICU, the SAPS 3 severity score was not associated with CRP values, but with DNA damage, only after 72 hours of ICU admission. It is important to know the severity and clinical characteristics of ICU patients to develop a care plan based on the peculiarities of the population attended.

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AUTHORS' CONTRIBUTIONS

Andriela Vieceli Mattje and Patrícia Molz contributed to the conception, design, analysis, and writing of the article;

Caio Fernando de Oliveira, Diene da Silva Schlickmann, Jane Dagmar Pollo Renner, Léo Kreater Neto, Daniel Prá, and Silvia Isabel Rech Franke contributed to the planning, design, revision, and final approval of the article;

All authors approved the final version of the manuscript and declared themselves responsible for all aspects of the work, guaranteeing their accuracy and integrity.

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