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sRAGE in septic shock: a potential biomarker of mortality

sRAGE no choque séptico: um potencial biomarcador de mortalidade

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ABSTRACT

Objective: To evaluate and understand the clinical implications of the plasma levels of a soluble isoform of a receptor for advanced glycation end products (sRAGE) in different stages of sepsis.

Methods: Serum sRAGE values in patients who were divided into intensive care unit control, severe sepsis, septic shock and recovery from septic shock groups were statistically analyzed to assess quantity (Kruskal-Wallis), variability (Levine test) and correlation (Spearman rank test) with certain inflammatory mediators (IL-1 α , IL-6, IL-8, IL-10, IP-10, G-CSF, MCP-1, IFN- γ and TNF- α).

Results: No changes in sRAGE levels were observed among the groups; however, the septic shock group showed

differences in the variability of sRAGE compared to the other groups. A positive correlation with all the inflammatory mediators was reported in the septic shock group.

Conclusion: sRAGE levels are associated with worse outcomes in patients with septic shock. However, a statistical correlation analysis with other proinflammatory cytokines indicated that the pathways leading to those outcomes are different depending on the sRAGE levels. Future studies to elucidate the pathophysiological mechanisms involving sRAGE in models of sepsis are of great clinical importance for the safe handling of this biomarker.

Keywords: Biological markers; Inflammation; Sepsis; Shock, septic; Survival

Conflicts of interest: None.

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INTRODUCTION

Sepsis is directly associated with severe systemic inflammatory responses. One of the mechanisms by which infection contributes to the presence of the persistent inflammatory response pathway is mediated by receptor-ligand binding. The receptor for advanced glycation end products (RAGE) and toll-like receptors (TLRs) belong to the group of pattern recognition receptors.⁽¹⁾

Ligands for RAGE include AGEs, the S100 family of proteins, peptides, beta amyloid, HGMB1, MAC1 and LPS. The interaction between RAGE and its ligands is promoted and mediated by the transcription factor NF- κ B cascade, culminating in the increased production of inflammatory mediators. Similar to some members of the TLR family (TLR-2 and TLR-4), RAGE has a soluble isoform (sRAGE) that originates from alternative splicing of mRNA and/or proteolytic cleavage.^(2,3)

Currently, there is debate in the literature regarding the functions of sRAGE. Some researchers believe that sRAGE acts as a decoy receptor, hindering the increase of pro-inflammatory mediators, whereas other researchers relate sRAGE with propagation of the inflammatory response by binding to CD11b receptors on leukocytes.^(4,5) The differing opinions are justified in part because of the lack of published data regarding sRAGE. To help elucidate the functions of sRAGE, this study aimed to evaluate the correlation between plasma levels of sRAGE, the inflammatory response and survival in patients with varying degrees of sepsis.

METHODS

Study design

This study was a prospective cohort study conducted at one of the *Hospital das Clínicas* Intensive Care Units (*Universidade de São Paulo*, Brazil) and was conceived as part of the BRISK Project (Brazilian Initiative for Sepsis Knowledge), which was launched in 2009 to investigate many molecular aspects of sepsis. Surgical, trauma and coronary syndrome patients are usually admitted to other intensive care units (ICU) than that in our hospital, which makes our population very homogeneous. Indeed, the following five reasons account for more than 90% of the ICU admissions included in this study: sepsis, stroke, an altered level of consciousness, pulmonary edema, and asthma/chronic obstructive pulmonary disease (COPD).

Patients who were less than 18 years old, pregnant, with disseminated malignancies or receiving chemotherapy, HIV-positive, with advanced hepatic disease, in end-of-life conditions, and those who refused to participate in the study were excluded. The remaining patients were divided in four groups: (1) ICU control (patients admitted to the ICU for noninfectious causes); (2) severe sepsis group (patients admitted to the ICU for severe sepsis or who developed severe sepsis during the ICU stay); (3) septic shock group (patients admitted to the ICU for septic shock or who developed septic shock during the ICU stay); and (4) recovery from shock septic group (patients who recovered from septic shock). Data and blood samples were collected at the patients' admission or when severe sepsis and septic shock were diagnosed by the medical staff. Severe sepsis and septic shock were defined as proposed in 1992 according to the American College of Chest Physicians/Society of Critical Care Medicine (ACCP/SCCM) Consensus Conference Committee.⁽⁶⁾ The

serum sRAGE and inflammatory mediators were analyzed using the MILLIPLEX[®] MAP kits based on Luminex[®] Technology (EMD Millipore, Massachusetts, USA).

Statistical analysis

Three different statistical methods were used: (1) the Kruskal-Wallis test (used to compare the amount of plasma sRAGE between the ICU control, severe sepsis, septic shock and recovery from septic shock groups); (2) the Levine test (used to compare the variability of plasma sRAGE between the ICU control, severe sepsis, septic shock and recovery from septic shock groups); and (3) the Spearman test (used to identify the relevance of the correlation between levels of sRAGE and inflammatory mediators in different contexts).

RESULTS

The Kruskal-Wallis test showed no distinction between plasma levels of sRAGE in patients with different degrees of sepsis; however, the variability analysis (Levine test) indicated that the group of patients with septic shock was significantly different compared to the other groups (Figure 1). The differences observed in the analysis of variability can be explained in part by evaluating the correlation between plasma levels of sRAGE with the values of some inflammatory mediators.

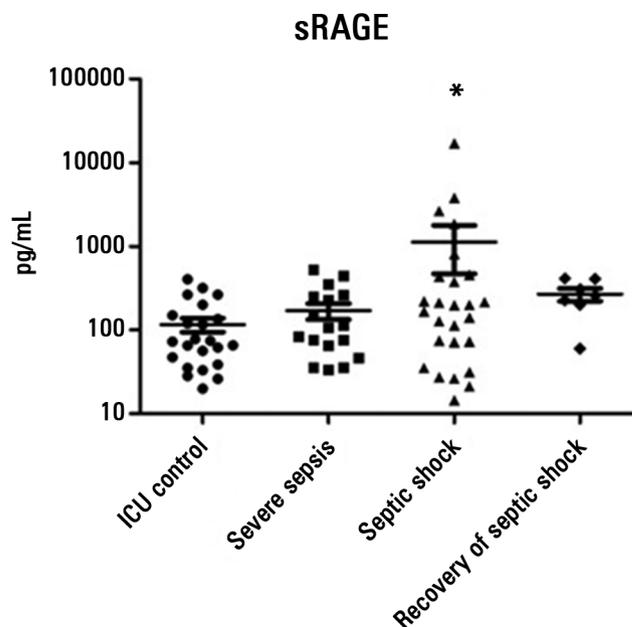


Figure 1 - Serum levels (pg/mL) of sRAGE in different stages of sepsis. The level of sRAGE in the septic shock group exhibited significant variation, which was detected by the Levine test (represented in the graph by the * symbol). ICU - intensive care unit.

A positive correlation between tumor necrosis factor alpha (TNF- α) and sRAGE was observed in all the groups except in the group of patients who recovered from septic shock. There was a positive correlation between interferon gamma (IFN- γ) and sRAGE in conditions of severe sepsis and septic shock. Patients who developed septic shock and those who recovered from this condition showed a positive correlation between levels of sRAGE and monocyte chemoattractant protein-1 (MCP-1) and between sRAGE and *granulocyte colony stimulating factor* (G-CSF). Interleukin (IL)-1 α , IL-6, IL-8, IL-10 and interferon-gamma-induced-protein 10 (IP-10) showed a positive and statistically significant correlation with sRAGE only in patients with septic shock (Table 1).

Depending on the results from the variability and correlation tests, the assessment of survival focused on the group of patients who developed septic shock. High levels (greater than 500pg/mL) of sRAGE were found in five patients, with four (80%) patients dying. Decreased levels of sRAGE (less than 100pg/mL) were found in seven patients, with five (71%) patients dying. Of all the patients included in the septic shock group (n=26), 46.1% (n=12) of those patients died in the ICU. Additionally, 75% (n=9) of patients who died had either elevated or decreased sRAGE levels. These results indicate that increased and decreased plasma levels of sRAGE (together) represent a sensitivity of 75%, a specificity of 85%, a positive-predictive value of 75% and a negative-predictive value of 85% in relation to the prognosis of death in patients with septic shock.

To verify the degree of correlation between sRAGE and inflammatory mediators associated with patient survival, the septic shock group was divided for further correlation analyses in patients with septic shock who survived and patients who died. With the exception of the chemokine

MCP-1, which shared a significant positive correlation between the two groups evaluated, every other inflammatory mediator (IL-1 α , IL-6, IL-8, IL-10, IP-10, G-CSF, IFN- γ and TNF- α) showed a significant positive correlation only in the group of patients who died (Table 2).

To understand which inflammatory mediators were associated with death under increased (greater than 500pg/mL) or reduced (less than 100 pg/mL) concentrations of sRAGE, a correlation analysis was performed. sRAGE showed significant positive correlations with IL-1 α , IFN- γ and TNF- α in patients who showed an increase in sRAGE levels. In contrast, the group of patients with decreased sRAGE levels showed no significant correlations with any of the inflammatory mediators investigated (Table 2).

DISCUSSION

The signaling cascades and ligands for RAGE in the literature suggest a fundamental role of this receptor in the inflammatory process. For this reason, studies have associated RAGE with various pathologies.⁽⁷⁾

The first published work regarding increased plasma levels of sRAGE from patients with sepsis was relatively recent and was reported by Bopp et al.⁽⁸⁾ In that study, patients with severe sepsis and septic shock (combined in a single group) showed an increase of sRAGE levels in plasma compared to healthy controls. In addition, septic patients who died had increased plasma levels of sRAGE compared to patients who survived. Similar data relating increased plasma levels of sRAGE to death in septic patients have recently been described.⁽⁹⁾

Although the groups were organized differently, the results of the current study do not demonstrate the same aspects described by Bopp et al.⁽⁸⁾ Initially, no statistically significant differences were observed in the plasma levels

Table 1 - Correlation analysis with sRAGE at different stages of sepsis

	ICU control (N=23)		Severe sepsis (N=17)		Septic shock (N=26)		Recovery of septic shock (N=7)	
	r	p value	r	p value	r	p value	r	p value
IL-1 α	0.34	>0.05	0.37	>0.05	0.37	$\leq 0.05^*$	0.03	>0.05
IL-6	0.09	>0.05	0.08	>0.05	0.59	$\leq 0.01^*$	0.17	>0.05
IL-8	0.13	>0.05	0.01	>0.05	0.58	$\leq 0.01^*$	0.07	>0.05
IL-10	0.31	>0.05	0.13	>0.05	0.46	$\leq 0.01^*$	-0.07	>0.05
G-CSF	0.04	>0.05	0.02	>0.05	0.51	$\leq 0.01^*$	0.85	$\leq 0.05^*$
IFN- γ	0.46	>0.05	0.58	$\leq 0.05^*$	0.44	$\leq 0.05^*$	-0.42	>0.05
IP-10	0.44	>0.05	0.39	>0.05	0.46	$\leq 0.01^*$	-0.07	>0.05
MCP-1	0.01	>0.05	0.14	>0.05	0.76	$\leq 0.001^*$	0.82	$\leq 0.05^*$
TNF- α	0.47	$\leq 0.05^*$	0.47	$\leq 0.05^*$	0.56	$\leq 0.01^*$	0.46	>0.05

ICU - intensive care unit; IL - interleukin; G-CSF - granulocyte colony stimulating factor; IFN- γ - interferon gamma; IP-10 - interferon-gamma-induced-protein 10; MCP-1 - monocyte chemoattractant protein-1; TNF- α - tumor necrosis factor alpha. * p values ≤ 0.05 .

Table 2 - Correlation analysis with sRAGE in patients with septic shock

	Survivors (N=14)				Non-survivors (N=12)			
	Total		Total		With increase of sRAGE (N=4)		With decrease of sRAGE (N=5)	
	r	p value	r	p value	r	p value	r	p value
IL-1 α	0.18	>0.05	0.58	$\leq 0.05^*$	0.96	$\leq 0.05^*$	0.10	>0.05
IL-6	0.30	>0.05	0.76	$\leq 0.01^*$	0.38	>0.05	0.70	>0.05
IL-8	0.46	>0.05	0.65	$\leq 0.05^*$	0.43	>0.05	0.30	>0.05
IL-10	0.05	>0.05	0.77	$\leq 0.01^*$	-0.24	>0.05	0.30	>0.05
G-CSF	0.04	>0.05	0.70	$\leq 0.01^*$	-0.19	>0.05	0.40	>0.05
IFN- γ	0.11	>0.05	0.70	$\leq 0.01^*$	0.99	$\leq 0.01^*$	-0.20	>0.05
IP-10	0.44	>0.05	0.69	$\leq 0.05^*$	0.69	>0.05	0.30	>0.05
MCP-1	0.64	$\leq 0.05^*$	0.77	$\leq 0.01^*$	0.24	>0.05	0.30	>0.05
TNF- α	0.18	>0.05	0.81	$\leq 0.01^*$	0.98	$\leq 0.01^*$	0.50	>0.05

IL - interleukin; G-CSF - granulocyte colony stimulating factor; IFN- γ - interferon gamma; IP-10 - interferon-gamma-induced-protein 10; MCP-1 - monocyte chemoattractant protein-1; TNF- α - tumor necrosis factor alpha. * p values ≤ 0.05 .

of sRAGE between different degrees of sepsis (severe sepsis and septic shock), even when compared between individuals who entered the ICU for noninfectious reasons or individuals who were recovering from septic shock. No statistically significant differences between patients who survived and patients who died while in septic shock were observed, according to the Student's *t*-test.

Our results indicate a statistically significant difference between sRAGE variability of the septic shock group and the other groups (Figure 1). It is possible that decreases in sRAGE blood levels may be associated with increased mortality in septic shock.

The results from the correlation analysis between sRAGE and inflammatory mediators in the different groups (Table 1) suggest that the positive correlation between TNF- α and sRAGE may be an important factor in the process that develops from ICU admission to the manifestations of septic shock because only the patients who recovered from septic shock showed no statistically significant correlation in this analysis. Similarly, the positive correlation between IFN- γ and sRAGE may consist of a difference between the group of individuals in the ICU group and the group that developed severe sepsis. Finally, significant correlations between sRAGE and IL-1 α , IL-6, IL-8, IL-10 and IP-10 were found only in the septic shock group.

Plasma levels of sRAGE appear to be associated with their ligands. An *in vitro* study by Raucci et al.⁽¹⁰⁾ reported that when cleavage occurs, overall RAGE levels increase; likewise, the amount of sRAGE in the supernatant of cells treated with HMGB1 (one of the ligands for RAGE)

increases. Similar results from a study on patients who had elevated blood levels of AGE were observed.⁽¹¹⁾

It has been reported that an increased level of sRAGE would be a protective mechanism because its presence in the plasma contributes to the removal or neutralization of ligands for RAGE, thereby acting as a decoy receptor.⁽⁴⁾ However, Wang et al.⁽⁵⁾ reported a deleterious effect of sRAGE in the inflammatory process because it would bind to CD11b receptors on leukocytes and propagate inflammation.

Our results on the statistical correlation between the total number of patients who survived and all the patients who died (Table 2) indicated that patients who died showed a positive correlation between sRAGE and several pro-inflammatory mediators (IL-1 α , IL-6, IL-8, IP-10, IFN- γ and TNF- α).

In the group of patients with increased sRAGE levels (greater than 500pg/mL) who died, positive correlations between sRAGE and IL-1 α , IFN- γ , and TNF- α were observed (Table 2). These results are consistent with those proposed by Wang et al.⁽⁵⁾, suggesting that during septic shock, elevated serum levels of sRAGE appear to be associated with worse outcomes via pathways related to IL-1 α , IFN- γ and TNF- α .

Surprisingly, we observed that decreased levels of sRAGE in the plasma (less than 100pg/mL) may also be related to mortality levels. However, we could not find any correlation with the inflammatory mediators investigated. Thus, further studies are required to elucidate the pathways related to the decreased levels of sRAGE that lead to a worse outcome.

CONCLUSION

We observed that increased levels of sRAGE are associated with worse outcomes in patients with septic shock; likewise, reduced sRAGE levels are also associated with increased mortality. However, the correlation analysis indicated that the pathways leading to death that are associated with increased or decreased sRAGE plasma levels are different. Future studies to elucidate the pathophysiological mechanisms triggered by sRAGE in models of sepsis are of great importance for the safe handling of this biomarker.

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RESUMO

Objetivo: Avaliar e compreender as implicações clínicas dos níveis plasmáticos de uma isoforma solúvel de um receptor de produtos finais de glicação avançada (do inglês *receptor for advanced glycation end products* - sRAGE) em diferentes fases da sepse.

Métodos: Os valores do sRAGE sérico em pacientes divididos nos grupos controle na unidade de terapia intensiva, sepse grave, choque séptico e recuperação de choque séptico foram analisados do ponto de vista estatístico para avaliar a quantidade (Kruskal-Wallis), variabilidade (teste de Levine) e correlação (teste Spearman rank) em relação a certos mediadores inflamatórios (IL-1 α , IL-6, IL-8, IL-10, IP-10, G-CSF, MCP-1, IFN- γ e TNF- α).

Resultados: Não se observaram modificações nos níveis de sRAGE entre os grupos; contudo o grupo com choque

séptico demonstrou diferenças na variabilidade do sRAGE em comparação aos demais grupos. Foi relatada, no grupo com choque séptico, uma correlação positiva com todos os mediadores inflamatórios.

Conclusão: Os níveis de sRAGE se associaram com desfechos piores nos pacientes com choque séptico. Entretanto, uma análise de correlação estatística com outras citocinas pró-inflamatórias indicou que as vias que levam a esses desfechos são diferentes, dependendo dos níveis de sRAGE. A realização de estudos futuros para elucidar os mecanismos fisiopatológicos que envolvem sRAGE nos modelos de sepse será de grande importância clínica para possibilitar o uso seguro desse biomarcador.

Descritores: Marcadores biológicos; Inflamação; Sepse; Choque séptico; Sobrevida

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