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Soluble RAGE and the RAGE ligands HMGB1 and S100A12 in critical illness: impact of glycemic control with insulin and relation with clinical outcome

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Running head:
Soluble RAGE and RAGE ligands in critical illness.
ABSTRACT

**Purpose:** Systemic inflammation often leads to complications in critically ill patients. Activation of the receptor for advanced glycation end-products (RAGE) generates inflammatory cytokines, proteases and oxidative stress and may link inflammation to subsequent organ damage. Furthermore, hyperglycemia-induced oxidative stress increases RAGE ligands and RAGE expression. We hypothesized that preventing hyperglycemia during critical illness reduces the risk of excessively enhanced RAGE signaling which could relate to clinical outcomes and risk of death.

**Methods:** In 405 long-stay surgical ICU patients randomized to intensive or conventional insulin treatment, serum concentrations of soluble RAGE (decoy receptor) and the RAGE ligands high-mobility group box 1 (HMGB1) and S100A12 were measured on admission, day 7 and last day. These were compared with levels in 71 matched control subjects and with C-reactive protein (CRP) as routinely monitored inflammation marker.

**Results:** Upon admission, soluble RAGE, HMGB1, S100A12 and CRP were higher in patients than in controls. HMGB1, S100A12 and CRP remained elevated throughout ICU stay, whereas soluble RAGE decreased to levels lower than in controls by day 7. Unexpectedly, insulin treatment did not affect the circulating levels of these markers. In univariable analysis, elevated levels of soluble RAGE upon admission were associated with adverse outcome, including circulatory failure, kidney failure, liver dysfunction and mortality. The associations with circulatory and kidney failure remained significant in multivariable logistic regression analysis corrected for baseline risk factors.

**Conclusions:** Critical illness affects components of RAGE signaling, unaffected by insulin treatment. Elevated upon-admission soluble RAGE was associated with adverse outcomes.

**KEYWORDS**

Critical illness, inflammation, RAGE, sRAGE, HMGB1, S100A12
INTRODUCTION

Persistent systemic inflammation caused by infectious and non-infectious (trauma and surgery) conditions is frequently associated with immune suppression and catabolism and may ultimately lead to organ damage, neuromuscular weakness, prolonged dependency on intensive care and death [1]. The receptor for advanced glycation end-products (RAGE) has been identified as an important mediator amplifying and perpetuating inflammation, linking inflammation to subsequent organ damage and adverse outcome in e.g. sepsis, acute lung injury and myocardial dysfunction [2].

Engagement of membrane-bound RAGE activates several signaling pathways that regulate processes of inflammation, apoptosis, proliferation and autophagy. Among them, activation of the nuclear factor-kappa B (NF-kB)-pathway generates a wide array of inflammatory cytokines, chemokines, adhesion molecules and proteases, and increases the oxidative stress burden. Moreover, NF-kB activation leads to receptor up-regulation, which ensures perpetuation and amplification of the signal [3].

RAGE recognizes different ligands through their three-dimensional structure and has therefore been considered a pattern recognition receptor [3]. Putative ligands comprise advanced glycation end-products (AGEs), high-mobility group box 1 (HMGB1), S100 proteins, amyloid-ß-peptide and ß-fibrils [3]. Different soluble RAGE forms exist, probably acting as decoy receptor by binding the same ligands and thus conferring protection against overwhelming inflammation. sRAGE is a truncated form of RAGE, which under inflammatory conditions is cleaved from the full-length receptor by A-disintegrin and metalloprotease 10 (ADAM10) [4]. Endogenous secreted RAGE (esRAGE) is produced by alternative mRNA splicing [5]. The nuclear DNA-binding protein HMGB1 is actively secreted by activated inflammatory cells or passively released by necrotic cells in areas of tissue damage. Extracellular functions relate to neurite outgrowth, cell migration, chemotaxis and tumor cell metastasis, dendritic cell maturation and activation of innate immune cells to release pro-inflammatory mediators. HMGB1 binds, amongst others, to RAGE, thus activating NF-kB and mitogen-activated protein kinase pathways [6]. HMGB1 has been described as a late mediator of sustained systemic inflammation in models of endotoxemia, peritonitis by cecal ligation and puncture and hemorrhagic shock [7, 8]. S100A12 is an intracellular calcium binding protein of the S100 family which is important in cell homeostasis. Upon cell damage, infectious or inflammatory signals, S100A12 is released from activated neutrophils into the
extracellular compartment and acts as a pro-inflammatory danger signal. Subsequent engagement of RAGE was demonstrated in e.g. processes of wound healing, tumor growth and inflammation [9].

Soluble RAGE (hereafter for convenience denoted as sRAGE) and RAGE-ligands have been evaluated as biomarkers of sustained or overwhelming inflammation predicting outcome in small studies on selected acute conditions as sepsis, acute respiratory distress syndrome (ARDS) and pneumonia [10, 11]. They have also been related to prognosis, development of complications and guiding of therapy in chronic inflammatory and autoimmune diseases, such as coronary artery disease, rheumatoid arthritis and diabetes [12]. In particular, in diabetes hyperglycemia-induced acceleration of RAGE ligand production has been related to RAGE activation, increased production of reactive oxygen species (ROS) and diabetic complications, whereas RAGE ligands and soluble RAGE are modulated with glycemic control [13, 14].

In this study, we quantified the circulating levels of sRAGE, HMGB1 and S100A12 at different time points in a large heterogeneous group of long-stay critically ill patients. In view of the common development of hyperglycemia in critical illness and the highlighted diabetes literature, we hypothesized that maintaining normoglycemia with intensive insulin therapy (IIT) during ICU stay [15] would attenuate the levels of HMGB1 and S100A12 while increasing sRAGE levels. As mentioned, data on the levels of these markers were only available from small, selected subgroups of critically ill patients. Therefore, we also performed a detailed assessment of whether admission levels of these proteins in a large heterogeneous patient population are associated with baseline characteristics of the patients or clinical complications. Baseline characteristics included type of surgery, sepsis upon admission, medical history, body mass index and age. The acquisition of a bloodstream infection, development of organ dysfunction (circulatory failure, kidney and liver dysfunction) and mortality were studied as clinical outcome variables. In addition, we systematically compared the performance of these markers with C-reactive protein (CRP) as a routinely measured inflammation marker.
MATERIALS AND METHODS

Subjects
This study is a pre-planned subanalysis of patients enrolled in a large prospective, randomized, controlled study on the effects of maintaining normoglycemia with intensive insulin therapy on outcome in a university hospital surgical intensive care unit [15]. In brief, strict glycemic control (80-110 mg/dl) with continuous infusion of insulin (intensive insulin therapy, IIT) was compared with treating stress hyperglycemia only when exceeding 215 mg/dl and stopping insulin infusion when levels dropped below 180 mg/dl (conventional insulin therapy, CIT). All patients requiring at least 7 days of intensive care (n=405) were included in this analysis (Table 1). We included 71 healthy individuals as a control group, matched with the patients for age (65 [55-73] years, \( P = 0.4 \)), gender (69% male, \( P = 0.8 \)) and BMI (25.5 [23.3-27.3] kg/m^2, \( P = 0.8 \)).

Written informed consent was obtained from the volunteers, the patients or their next-of-kin. The study protocol, as well as the consent forms, were approved by the KU Leuven Institutional Review Board (ML1094, ML2707). The study was performed in accordance with the Declaration of Helsinki.

Blood sampling and biochemical analyses
Blood glucose and plasma CRP, creatinine, and bilirubin were determined with routine clinical chemistry assays during the clinical study. Additional blood samples were taken upon admission and daily at 6.00 a.m. until discharge or death. After centrifugation, serum was kept frozen at -80°C until analysis. We determined the concentration of sRAGE, HMGB1 and S100A12 in serum harvested upon admission, day 7 and last day in ICU, using commercial enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer’s instructions (Quantikine Human RAGE Immunoassay, R&D Systems, Abingdon, UK; HMGB1 ELISA, IBL, Hamburg, Germany; CircuLex S100A12/EN-RAGE ELISA kit, MBL International Corporation, Woburn, MA).

Statistical analysis
We used StatView 5.0.1 (SAS Institute Inc., Cary, NC) for statistical analyses. Data are presented as medians and interquartile ranges or as proportions and percentages. Continuous variables were analyzed with Mann-Whitney U and proportions with the chi-square test. Associations between variables were calculated with Spearman’s correlation coefficient. Multivariable regression analysis was performed to further assess the significant associations of baseline levels of sRAGE, HMGB1 and S100A12 with ICU outcomes (acquisition of
a bloodstream infection, circulatory failure, kidney dysfunction, liver dysfunction and mortality) while correcting for baseline risk factors. Known classical baseline characteristics and risk factors (history of diabetes or malignancy, severity of illness, admission diagnosis, sepsis upon admission) as well as baseline CRP, as routinely measured inflammation marker, were considered, together with serum levels upon-admission of sRAGE, HMGB1 and S100A12, entered either separately or combined. Area under the receiver-operating characteristic (ROC) curves (AUC) for baseline levels of sRAGE, HMGB1, S100A12 and CRP in the prediction of outcome were calculated with JMP10.0.0 (SAS). Two-sided *P* values below 0.05 were considered significant. Sample numbers provided 100% statistical power to demonstrate a 50% increase in sRAGE and HMGB1 upon admission to the ICU as compared with healthy controls and a 25% change by IIT as compared with CIT [16].
RESULTS

*sRAGE, HMGB1, S100A12 and CRP in critically ill patients versus healthy controls*

Upon admission in ICU, serum sRAGE, HMGB1, S100A12 and CRP were elevated as compared to healthy control levels (Table 2). sRAGE decreased to levels lower than those seen in healthy volunteers by day 7 and remained low on the last day in ICU (Figure 1). HMGB1, S100A12 and CRP also decreased during ICU stay, but always remained higher than healthy control levels.

*Impact of intensive insulin therapy on sRAGE, HMGB1, S100A12 and CRP*

Admission serum concentrations of sRAGE, HMGB1, S100A12 and CRP were comparable for the CIT and IIT groups (Figure 2). IIT did not affect circulating levels of these proteins at day 7 or last day in ICU (Figure 2), irrespective of having pre-existing diabetes or not (data not shown), but lowered CRP.

*Association of baseline sRAGE, HMGB1, S100A12 and CRP with baseline characteristics*

Cardiac surgery patients had similar sRAGE, but lower HMGB1, S100A12 and CRP levels upon ICU admission as compared with patients undergoing another type of surgery (Figure 3). HMGB1 and S10012 remained lower in cardiac surgery patients on day 7, but were comparable on the last day in ICU. In contrast, sRAGE levels on day 7 and last day and CRP levels on the last day were higher in cardiac surgery than in non-cardiac surgery patients. Patients with sepsis upon admission had higher upon-admission HMGB1, S100A12 and CRP levels than those without, but comparable sRAGE (Figure 3). sRAGE on day 7 and last day, as well as last day CRP were lower in patients with sepsis.

A history of diabetes did not affect the levels of these proteins, except that HMGB1 levels on day 7 were higher than in patients without previously diagnosed diabetes (data not shown). sRAGE was significantly lower in patients with a history of malignancy upon admission (1472 [763-2730] pg/ml) than in those without (1963 [1147-3151] pg/ml, \( P=0.02 \)). This difference was maintained at the later time points. None of the proteins were significantly associated with age or obesity (data not shown). There was no association between baseline sRAGE, HMGB1, S100A12 and CRP (data not shown).
Association of baseline sRAGE, HMGB1, S100A12 and CRP with infection, organ dysfunction and outcome

In univariable analysis, upon admission serum concentrations of sRAGE, HMGB1, S100A12 and CRP, or the time courses of these analytes, were not associated with subsequent acquisition of bacteremia (Figure 4). In contrast, admission sRAGE levels were higher in patients with circulatory failure defined as need of vasoactive support (1.96 [1.12-3.24] ng/ml) versus those without (1.42 [0.79-2.59] ng/ml, \(P=0.006\)). Admission sRAGE levels were also higher in patients who developed acute kidney injury, reflected by a peak creatinine exceeding 2.5 mg/dl (2.06 [1.35-2.98] ng/ml versus 1.76 [0.97-3.04] ng/ml, \(P=0.04\)) and by the need for renal replacement therapy (2.38 [1.50-3.47] ng/ml versus 1.71 [0.97-2.87] ng/ml, \(P=0.002\)) (Figure 4). Likewise, admission sRAGE was significantly elevated in patients who subsequently developed cholestatic liver dysfunction defined by a peak bilirubin \(\geq 2\) mg/dl (2.11 [1.23-3.47] ng/ml) versus patients with a lower peak bilirubin (1.55 [0.93-2.62] ng/ml, \(P=0.001\)) (Figure 4). Elevated admission sRAGE was also associated with hospital mortality (2.11 [1.43-3.52] ng/ml in non-survivors versus 1.80 [0.98-2.85] ng/ml in survivors, \(P=0.03\)). For renal replacement therapy and hospital mortality, these associations were still present on day 7 and last day in ICU (Figure 4).

Association of outcomes with the other inflammation markers upon ICU admission were limited to lower HMGB1 levels in patients who died in the hospital (4.84 [3.10-8.40] ng/ml in non-survivors versus 6.50 [3.48-11.19] ng/ml in survivors, \(P=0.02\)), and higher S100A12 levels in patients developing circulatory failure (0.68 [0.27-1.67] ng/ml versus 0.40 [0.13-1.16] ng/ml, \(P=0.01\)) or cholestatic liver dysfunction (0.79 [0.27-1.81] \(\mu\)g/ml versus 0.48 [0.18-1.32] \(\mu\)g/ml, \(P=0.01\)). These associations were less preserved throughout ICU stay. CRP levels on day 7 and last day in ICU were higher in patients who needed renal replacement therapy, in patients who developed cholestatic liver dysfunction and in patients who died in the hospital (Figure 4).

In multivariable regression analysis, correcting for baseline characteristics and risk factors (history of diabetes or malignancy, APACHE-II, type of critical insult and sepsis upon admission) as well as baseline CRP, elevated admission sRAGE was still associated with circulatory failure and need for renal replacement therapy (Table 3). Adding the admission values for HMGB1 and S100A12 to the model, sRAGE remained independently associated with need for renal replacement therapy. Interestingly, when the three inflammation markers were added to the model, S100A12 appeared independently associated with cholestatic liver dysfunction and HMGB1 with hospital mortality.

Nevertheless, ROC curve analysis showed poor predictive value of the markers in relation to outcome (data not shown).
DISCUSSION

We demonstrated that the decoy receptor sRAGE and the RAGE ligands HMGB1 and S100A12 are elevated in a large heterogeneous cohort of prolonged critically ill patients upon admission to ICU. sRAGE levels decreased below healthy control levels by day 7 and remained low until the last ICU day, whereas HMGB1 and S100A12 remained elevated. Maintenance of normal blood glucose levels with insulin unexpectedly did not affect the levels of these markers. Admission levels of sRAGE in particular were associated with subsequent organ dysfunction (circulatory dysfunction, need for renal replacement therapy and cholestatic liver dysfunction) and hospital mortality. These associations remained significant for circulatory and kidney failure after correction for baseline risk factors and severity of illness.

The elevated levels of sRAGE, HMGB1 and S100A12 upon ICU admission are in line with several studies in mostly small cohorts of critically ill patients, mostly with a selected pathology [10, 11, 17]. Interestingly, whereas admission sRAGE levels were elevated, the levels of sRAGE fell below the level of healthy volunteers at the later time points in ICU. This may reflect an acute peak induced by massive inflammation that induces a proteolytic environment and mediates receptor shedding, which is then followed by a sustained decrease that occurs when the condition becomes more chronic. The latter would thus reflect an exhausted protective anti-inflammatory system. Indeed, sRAGE probably acts as a decoy receptor by binding the RAGE ligands and may thus confer a protective mechanism against overwhelming inflammation. Decreased soluble RAGE levels have been reported in chronic conditions in which RAGE ligands accumulate, such as diabetes, atherosclerosis and rheumatoid arthritis, and where low levels of the soluble receptor are associated with more severe disease and worse outcome [18].

Critical illness is associated with inflammation and increased oxidative stress, contributing to organ failure and mortality. Binding of ligands to RAGE increases oxidative stress. In diabetes, hyperglycemia precipitates the pro-oxidant state, whereas glucose control is able to down-regulate RAGE signaling and the oxidative burden [13, 14, 19]. Several studies in patients with diabetes have indeed highlighted the association between glycemic control and level of sRAGE, RAGE ligands, markers of oxidative stress and diabetic complications. We previously demonstrated inhibition of excessive iNOS-induced NO release, possibly via reduced NF-kB-activation, in patients with prolonged critical illness [19] as well as reduced oxidative stress in critically ill
animals [20] with strict glycemic control. Thus, it was rather unexpected that the intervention did not affect circulating levels of sRAGE, HMGB1 or S100A12. Recent findings in a small cohort suggested a decrease of sRAGE by insulin therapy in critically ill patients with diabetes, but not in those without [16]. In our study, however, IIT failed to affect sRAGE irrespective of pre-existing diabetes.

Elevated sRAGE and S100A12 levels upon ICU admission correlated with the need for hemodynamic support. So far, this association has not been described. Interestingly though, inflammation induced by activation of the RAGE axis has been linked to subsequent myocardial and vascular dysfunction [21, 22].

sRAGE levels upon ICU admission were higher in patients who subsequently developed new kidney injury than in those who did not, whereas no such relation was found for the other markers. sRAGE was associated with new kidney injury independent of baseline risk factors and of the routinely measured inflammation marker CRP and the other studied markers. After severe trauma, a significant relationship has been observed between sRAGE and development of acute renal failure [23]. In patients with septic acute kidney injury, the kidney is regarded both culprit and target of RAGE signaling [24]. Actually, elevated levels of AGEs and sRAGE, accumulating due to impaired clearance as in chronic kidney disease [12, 25], lead to further activation and shedding of RAGE from the renal (mesangial) cells [24]. In our study, the acute increase in sRAGE is more likely to reflect the inflammatory burden as the vast majority of patients had no impairment of renal function prior to admission. Whereas we did not find any association of HMGB1 with acute kidney injury, HMGB1 is released from renal cells into the circulation after ischemia-reperfusion injury, inducing pro-inflammatory cytokine production. Neutralizing HMGB1 with antibodies or inhibiting its release reduced ischemic damage [26]. Data on S100A12 in relation to acute kidney injury are not available, but levels are known to be elevated in chronic kidney injury requiring hemodialysis [27]. Overall, accumulation of RAGE ligands, inducing RAGE up-regulation, has been proposed to contribute to both diabetic and non-diabetic nephropathy [13, 14].

Elevated sRAGE was also associated with the development of cholestatic liver dysfunction. Since the liver represents the major site of AGE metabolism, it is conceivable that impaired hepatic function might result in elevated levels of AGEs with subsequent RAGE activation. Moreover, high levels of RAGE expression are commonly observed in the biliary system [28], pointing at its potential role in the disposition of AGEs. The hepatocytes seem to be most vulnerable to the detrimental effects of AGEs [28]. Decreased sRAGE levels have
been described in liver steatosis [29], non-alcoholic fatty liver disease [30] and intrahepatic cholestasis of pregnancy [31]. These data support a role for sRAGE as decoy receptor and exhaustion of this defense mechanism in chronic conditions. No data are available on association of HMGB1 or S100A12 with cholestatic liver dysfunction of critical illness.

The association of elevated admission sRAGE and subsequent mortality is consistent with several previous studies. Thus, sRAGE predicted mortality in critically ill patients with sepsis, acute lung injury/ARDS and trauma [11, 21, 32], although not all studies found such association [16]. Whereas HMGB1 has been identified as a late mediator of sepsis-induced lethality in animal models, we observed lower HMGB1 levels upon ICU admission in patients who would not survive than in survivors. This counterintuitive finding has also been observed in patients with sepsis [33]. In contrast, elevated baseline HMGB1 predicted mortality in patients with pneumonia-related ARDS [34]. S100A12 has been linked to mortality and adverse outcome in patients with chronic renal failure and chronic cardiovascular disease [9, 27, 35], but data in critical illness are lacking.

In our study, elevated sRAGE was associated with organ dysfunction and death. However, caution is warranted regarding technical aspects of the assay used to quantify these levels. Several forms of soluble RAGE exist which may bind different ligands, whereas commercial ELISAs used in our study and most of the cited studies do not discriminate between these forms. Endogenous secreted RAGE (esRAGE) is produced by alternative mRNA splicing [5] and is regarded an endogenous anti-inflammatory decoy receptor, protecting from excessive inflammation by binding and scavenging potential ligands for RAGE. As such, elevated levels are believed to reflect an adequate, protective pathway. This may explain why elevated soluble RAGE levels are observed in extreme longevity, whereas decreased levels are associated with chronic (auto)immune and inflammatory diseases [12]. Another form, as mentioned, is released from the cell surface receptor under inflammatory conditions by ADAM10-mediated proteolytic cleavage and may represent (acute) RAGE activation and cellular damage, where the protective effects as decoy receptor are overwhelmed in a self-amplifying loop [4, 12]. Soluble RAGE levels measured in the present study may therefore reflect expression of the truncated receptor itself, the local proteolytic environment, or both. The source remains to be elucidated. In addition, this study merely established associations with outcome, but does not prove causality.
Conclusions

Critically ill patients show increased levels of soluble RAGE, HMGB1 and S100A12 upon ICU admission, likely reflecting massive inflammation. Soluble RAGE levels subsequently decreased below healthy control levels, which may reflect exhaustion of the protective anti-inflammatory system the decoy receptor provides, whereas HMGB1 and S100A12 remained elevated until the last day in ICU. Strict glycemic control during ICU stay had no impact on the levels of these markers and hence, these markers are not involved in the beneficial effects of this intervention on clinical outcome. In particular admission levels of soluble RAGE were associated with development of organ failure and with hospital mortality. Nevertheless, performance in ROC curve analysis was poor, indicating that the studied markers are not generally suitable as circulating biomarker to predict outcome of critically ill patients.
APPENDIX

LIST OF ABBREVIATIONS
ADAM10: A disintegrin and metalloprotease 10, AGEs: advanced glycation end-products, APACHE-II: acute physiology and chronic health-II score, ARDS: acute respiratory distress syndrome, BMI: body mass index, CIT: conventional insulin therapy, CRP: C-reactive protein, ELISA: enzyme-linked immunosorbent assay, HMGB1: high-mobility group box 1, ICU: intensive care unit, IIT: intensive insulin therapy, NF-kB: nuclear factor kappa B, RAGE: receptor for advanced glycation end-products, ROS: reactive oxygen species, sRAGE: soluble RAGE.

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### Table 1: Baseline characteristics

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<th>Conventional insulin therapy n=224</th>
<th>Intensive insulin therapy n=181</th>
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<tr>
<td>Sex (male, n (%))</td>
<td>150 (67)</td>
<td>125 (69)</td>
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<tr>
<td>Age (yr, median [IQR])</td>
<td>67 [52-74]</td>
<td>66 [53-73]</td>
<td>0.9</td>
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<tr>
<td>BMI (kg/m², median [IQR])</td>
<td>24.9 [22.4-27.7]</td>
<td>25.4 [22.6-29.2]</td>
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<tr>
<td>History of diabetes (n (%))</td>
<td>21 (9)</td>
<td>18 (10)</td>
<td>0.8</td>
</tr>
<tr>
<td>History of malignancy (n %)</td>
<td>50 (22)</td>
<td>42 (23)</td>
<td>0.8</td>
</tr>
<tr>
<td>APACHE-II first 24h, median [IQR]</td>
<td>12 [8-15]</td>
<td>11 [8-16]</td>
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<tr>
<td>TISS-28 score first 24h; median [IQR]</td>
<td>39 [33-45]</td>
<td>39 [35-45]</td>
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<tr>
<td>Admission blood glucose (mg/dl, median [IQR])</td>
<td>137 [115-165]</td>
<td>133 [109-163]</td>
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Table 2: Admission levels of sRAGE, HMGB1 and S100A12 in critically ill patients and healthy controls

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<th>Healthy controls</th>
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<tr>
<td>sRAGE (pg/ml, median [IQR])</td>
<td>1897 [1056-3026]</td>
<td>1515 [1177-1948]</td>
<td>0.03</td>
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<tr>
<td>HMGB1 (ng/ml, median [IQR])</td>
<td>6.3 [3.4-10.4]</td>
<td>2.8 [2.0-5.2]</td>
<td>&lt;0.0001</td>
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<td>S100A12 (ng/ml, median [IQR])</td>
<td>617 [234-1537]</td>
<td>112 [52-259]</td>
<td>&lt;0.0001</td>
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Table 3: Multivariable logistic regression analysis for RAGE-axis markers in relation to outcome

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<th>Outcome variable</th>
<th>Odds ratio (95% confidence interval)</th>
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<tr>
<td>Circulatory failure</td>
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<tr>
<td>A.  sRAGE (per 100 pg/ml added)</td>
<td>1.017 (1.001-1.033)</td>
<td>0.03</td>
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<tr>
<td>HMGB1 (per ng/ml added)</td>
<td>1.017 (0.986-1.050)</td>
<td>0.28</td>
</tr>
<tr>
<td>S100A12 (per 100 ng/ml added)</td>
<td>1.032 (1.005-1.060)</td>
<td>0.01</td>
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<tr>
<td>B.  sRAGE (per 100 pg/ml added)</td>
<td>1.015 (0.999-1.031)</td>
<td>0.06</td>
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<td>HMGB1 (per ng/ml added)</td>
<td>1.005 (0.969-1.043)</td>
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<tr>
<td>S100A12 (per 100 ng/ml added)</td>
<td>1.027 (0.996-1.058)</td>
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<td>Renal replacement therapy</td>
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<td>A.  sRAGE (per 100 pg/ml added)</td>
<td>1.012 (1.000-1.024)</td>
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<tr>
<td>HMGB1 (per ng/ml added)</td>
<td>0.998 (0.971-1.025)</td>
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<td>S100A12 (per 100 ng/ml added)</td>
<td>1.009 (0.985-1.033)</td>
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<tr>
<td>B.  sRAGE (per 100 pg/ml added)</td>
<td>1.013 (1.001-1.025)</td>
<td>0.03</td>
</tr>
<tr>
<td>HMGB1 (per ng/ml added)</td>
<td>0.986 (0.955-1.019)</td>
<td>0.39</td>
</tr>
<tr>
<td>S100A12 (per 100 ng/ml added)</td>
<td>1.011 (0.984-1.038)</td>
<td>0.44</td>
</tr>
<tr>
<td>Cholestatic liver dysfunction</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A.  sRAGE (per 100 pg/ml added)</td>
<td>1.010 (1.000-1.020)</td>
<td>0.06</td>
</tr>
<tr>
<td>HMGB1 (per ng/ml added)</td>
<td>0.992 (0.971-1.014)</td>
<td>0.48</td>
</tr>
<tr>
<td>S100A12 (per 100 ng/ml added)</td>
<td>1.016 (0.997-1.035)</td>
<td>0.10</td>
</tr>
<tr>
<td>B.  sRAGE (per 100 pg/ml added)</td>
<td>1.009 (0.998-1.019)</td>
<td>0.10</td>
</tr>
<tr>
<td>HMGB1 (per ng/ml added)</td>
<td>0.979 (0.954-1.005)</td>
<td>0.10</td>
</tr>
<tr>
<td>S100A12 (per 100 ng/ml added)</td>
<td>1.024 (1.002-1.047)</td>
<td>0.03</td>
</tr>
<tr>
<td>Hospital mortality</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A.  sRAGE (per 100 pg/ml added)</td>
<td>1.007 (0.995-1.018)</td>
<td>0.25</td>
</tr>
<tr>
<td>HMGB1 (per ng/ml added)</td>
<td>0.971 (0.939-1.004)</td>
<td>0.08</td>
</tr>
<tr>
<td>S100A12 (per 100 ng/ml added)</td>
<td>1.000 (0.977-1.023)</td>
<td>0.97</td>
</tr>
<tr>
<td>B.  sRAGE (per 100 pg/ml added)</td>
<td>1.007 (0.995-1.019)</td>
<td>0.24</td>
</tr>
<tr>
<td>HMGB1 (per ng/ml added)</td>
<td>0.961 (0.925-0.999)</td>
<td>0.04</td>
</tr>
<tr>
<td>S100A12 (per 100 ng/ml added)</td>
<td>1.012 (0.986-1.040)</td>
<td>0.37</td>
</tr>
</tbody>
</table>

Multivariable logistic regression models were built including the classical baseline risk factors history of diabetes, history of malignancy, cardiac versus non-cardiac surgery, severity of illness (APACHE-II score), sepsis upon admission, as well as CRP as classical marker of inflammation. The RAGE-axis markers were added to the model either separately (A) or all combined (B).
FIGURE LEGENDS

**Fig 1.** Levels of sRAGE, HMGB1, S100A12 and CRP during ICU stay
Protein levels on admission, day 7 and last day of ICU stay are shown and are compared to the levels in healthy individuals (grey lines represent median and interquartile range). Data are expressed as boxplots, with the central lines indicating the median, the boxes the interquartile range and the whiskers the 10th and 90th percentile. \( P \)-values were calculated with Mann-Whitney U. Numbers of patients available for analysis are indicated below the graphs.

**Fig 2.** Effect of strict glycemic control with intensive insulin therapy on sRAGE, HMGB1, S100A12 and CRP.
Protein levels on admission, day 7 and last day of ICU stay are compared for patients who received conventional (CTT) or intensive insulin therapy (IIT) during ICU stay. Data are presented as boxplots, with the central lines indicating the median, the boxes the interquartile range and the whiskers the 10th and 90th percentile. \( P \)-values were calculated with Mann-Whitney U. Numbers of patients available for analysis in each group are indicated below the graphs.

**Fig 3.** Levels of sRAGE, HMGB1, S100A12 and CRP in relation to baseline characteristics
Protein levels on admission, day 7 and last day of ICU stay are compared for patients with cardiac versus other surgery or having sepsis upon admission or not. Data are presented as boxplots, with the central lines indicating the median, the boxes the interquartile range and the whiskers the 10th and 90th percentile. \( P \)-values were calculated with Mann-Whitney U. Numbers of patients available for analysis in each group are indicated below the graphs.

**Fig 4.** Levels of sRAGE, HMGB1, S100A12 and CRP in relation to clinical outcome
Protein levels on admission, day 7 and last day of ICU stay are compared for patients with versus without bacteremia, circulatory failure, need for renal replacement therapy and cholestatic liver dysfunction as well as for patients who died in the hospital versus those who survived. Data are expressed as boxplots, with the central lines indicating the median, the boxes the interquartile range and the whiskers the 10th and 90th percentile. \( P \)-
values were calculated with Mann-Whitney U. Numbers of patients available for analysis in each group are indicated below the graphs.
Figure 1

Legend
Figure 2

Legend

- □ Conventional insulin therapy
- □ Intensive insulin therapy
Figure 3

Legend
Figure 4

Legend