Decrease in histidine-rich glycoprotein as a novel biomarker to predict sepsis among systemic inflammatory response syndrome

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ABSTRACT

Objective

Many biomarkers for sepsis are used in clinical practice, however, few have become the standard. We measured plasma histidine-rich glycoprotein (HRG) levels in patients with systemic inflammatory response syndrome (SIRS). We compared HRG, procalcitonin (PCT), and presepsin levels to assess their significance as biomarkers.

Design

Single center, prospective, observational cohort study.

Setting

Intensive Care Unit (ICU) at a university affiliated hospital.

Patients

Seventy-nine ICU patients (70 with SIRS and 9 without SIRS) and 16 healthy volunteers.

Interventions

None

Measurements and Main Results

We collected blood samples from patients within 24h of ICU admission. HRG levels were determined using enzyme-linked immunosorbent assay (ELISA). The median

HRG level in healthy volunteers (n=16) was 63.00 (interquartile range, 51.53–66.21) μg/ml. HRG levels in SIRS patients (n=70, 28.72 [15.74–41.46] μg/ml) were lower than those in non-SIRS patients (n=9, 38.64 [30.26–51.81] μg/ml; P = .049). Of 70 patients with SIRS, 20 had sepsis. HRG levels were lower in septic patients than in non-infective SIRS patients (8.71 [6.72–15.74] vs 33.27 [26.57–44.99] μg/ml; P < .001) and were lower in non-survivors (n=8) than in survivors (n=62) of SIRS (9.06 [4.49–15.70] vs. 31.78 [18.57–42.11] μg/ml; P < .001). HRG showed a high sensitivity and specificity for diagnosing sepsis. Receiver-operating characteristic (ROC) curve analysis for detecting sepsis within SIRS patients showed that the area under the curve for HRG, PCT, and presepsin was 0.97, 0.82, and 0.77, respectively. In addition, survival analysis in SIRS patients revealed that the Harrell C-index for HRG, PCT, and presepsin was 0.85, 0.65, and 0.87, respectively.

Conclusions

HRG levels were low in patients with sepsis and were significantly related to mortality in SIRS population. Moreover, as a biomarker, HRG may be superior to PCT and presepsin.

INTRODUCTION

Sepsis is a systemic illness, one of the most severe diseases of patients encountered in the intensive care unit (ICU). Despite recent medical progress, the mortality rate of patients with sepsis shows little sign of improvement (1, 2). There are many clinical biomarkers available for rapid diagnosis of sepsis; however, few have become standard (3). Procalcitonin (PCT), the soluble triggering receptor expressed on myeloid cells-1 (sTREM-1), and presepsin are considered promising diagnostic and prognostic biomarkers, although they are limited in their ability to diagnose sepsis (3, 4).

The liver produces histidine-rich glycoprotein (HRG) present in plasma. HRG is a multidomain (structured) protein that interacts with many ligands and is therefore thought to be involved in many functions such as coagulation, immune response, angiogenesis modulation, and others (5, 6). In particular, some articles previously reported that HRG, both in vitro and in vivo, was highly relevant to infections caused by bacteria (7) and fungi (8), and suggested that HRG plays a protective role in the host defense mechanism (7–9). Recently, our group demonstrated that HRG at normal physiological concentrations maintains circulating neutrophils and vascular endothelial cells quiescent, and that plasma HRG levels are decreased rapidly in mice with sepsis, triggering a cascade of events in septic pathogenesis including immunothrombosis,

acute respiratory distress syndrome (ARDS), and disseminated intravascular coagulation (DIC) (10). Based on these findings, we suggested a supplementary therapy with HRG for the treatment of sepsis (10).

In this study, we developed a new enzyme-linked immunosorbent assay (ELISA) to measure HRG levels in plasma and used it to perform a prospective observational study of patients with systemic inflammatory response syndrome (SIRS). We aimed to determine whether there was a difference between HRG levels of patients with and without infection as well as survivors and non-survivors.

MATERIALS and METHODS

Study design

We conducted a single-center, prospective, and observational investigation that was approved by the Institutional Review Board of the Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences. We followed guidelines as outlined in Strengthening the Reporting of Observational Studies in Epidemiology (11).

Patients and data collection

Patients newly admitted to the intensive care unit (ICU) of Okayama University Hospital were prospectively enrolled in the study if they fulfilled at least two diagnostic criteria for SIRS. Inclusion criteria were patients who were expected to stay in the ICU for >3 days (excluding less-severe patients) and those with an arterial blood collection line. Exclusion criteria were <20 years of age, pregnancy, only overnight stay in the ICU, or failure to obtain consent. For comparison, we collected blood samples from patients fulfilled all inclusion criteria except for SIRS criteria (non-SIRS ICU patients). In addition, plasma samples from healthy volunteers were collected and analyzed to determine HRG levels.

Clinical and laboratory data were collected daily while patients were in the ICU. Initial Sequential Organ Failure Assessment (SOFA) and Acute Physiology and Chronic Evaluation (APACHE) II scores were calculated using clinical parameters and blood test results. SIRS, sepsis, severe sepsis, and septic shock were classified according to the guidelines of the American College of Chest Physicians/Society of Critical Care Medicine and the International Surviving Sepsis Campaign Guidelines Committee (Sepsis-2) (12, 13). Follow-up investigations were conducted 28 and 90 days after enrollment and at ICU discharge to determine survivors and non-survivors.

Analytical methods

Blood samples were collected in tubes containing K_2EDTA (BD 367840; Beckton-Dickinson, Franklin Lakes, NJ, USA) within 24 h of ICU admission, processed within 30 min of sampling, and centrifuged at 3500 rpm for 10 min. The supernatant was pipetted into polypropylene tubes, a protease inhibitor cocktail (Complete mini EDTA-free; Roche Diagnostics, Basel, Switzerland) was added, and samples were stored at −80°C.

HRG levels were determined using the quantitative sandwich ELISA with a rat monoclonal antibody (mAb) against human HRG (made in-house, #75-14) as the capture antibody and horseradish peroxidase-conjugated nickel-nitrilotriacetic acid (Ni–NTA HRP Conjugate; Qiagen, Venlo, Netherlands) for detection. To perform ELISA, 3 µg of mAb per well was diluted in coating buffer (0.05M Na_2CO_3, pH 9.6), and immobilized on a 96-well plate (COSTAR 3590; Corning, Tewksbury, MA, USA) overnight at 4°C. After three washing steps using phosphate-buffered saline (PBS) containing 0.05% Tween 20, the plate was incubated with blocking buffer containing 3% bovine serum albumin (BSA) in PBS for 1 h at 37°C. After three further washing steps, plasma samples were diluted 1:50 in PBS containing 1% BSA and incubated for 2 h at 37°C in the mAb-coated wells on the microplate shaker set at 500 rpm. After three

washing steps, the plate was incubated with the Ni-NTA HRP conjugate diluted 1:1000 in PBS containing 0.2% BSA for 1.5 h at 37°C with shaking. After six extensive washing steps, o-phenylenediamine (Wako, Osaka, Japan) and stop solution (3M H_2SO_4) were added, and absorbance at 492 nm was measured using a 96-well plate reader (Model 680; Bio-Rad, Hercules, CA). A standard curve (Supplementary Fig. 2) was established using serial dilutions of known amounts of purified HRG (made in-house, Supplementary Content 1, Supplementary Fig. 3). Intra-assay reproducibility was determined by assaying the sample six times and inter-assay reproducibility was determined by five independent assays. The intra-assay and inter-assay coefficients of variability were 4.19% and 15.5%, respectively. Duplicate plasma samples were tested, and independent assays were repeated twice.

PCT levels were determined using an automated electrochemiluminescence immunoanalyzer (Modular Analytics E-170; Roche Diagnostics, Mannheim, Germany) in the Clinical Chemistry Laboratory of Okayama University Hospital. Presepsin levels were measured using PATHFAST Presepsin (LSI Medience, Tokyo, Japan).

Outcomes

The primary outcome of this study was to assess the significance of the difference

between HRG levels in healthy volunteers, non-SIRS patients, and patients with SIRS. Secondary outcomes were to assess differences between each marker in patients with and without sepsis as well as survivors and non-survivors of SIRS.

Statistical analysis

Data were expressed as median and interquartile ranges (IQR, 25th to 75th percentiles), all analyses were two-sided, and a P value < .05 was considered statistically significant. The Mann–Whitney test or the Kruskal–Wallis test implemented following the Steel–Dwass method was used to compare groups. The receiver-operating characteristic (ROC) curve analysis was used to determine the diagnostic accuracy. The Cox proportional hazard model and Kaplan–Meier method were used to analyze survival. We performed survival analysis using the 90–day mortality. In addition, we made adjustment with APACHE II score to correct for disease severity. When we performed Kaplan–Meier method, we divided patients into two groups according to the cut off value which was calculated in logistic regression model (sensitivity analysis); hazard ratio was calculated with Cox proportional hazard model. We calculated Spearman rank correlation coefficient to assess correlations between HRG and other parameters. We used JMP Pro 11 software (SAS Institute Inc., Chicago, IL, USA) for all analyses,

except for calculations of the Harrell C-index, which was determined using STATA 12 software (SAS Institute Inc.).

RESULTS

Patient characteristics

SIRS patients were prospectively included from November 2012 through November 2014. During this period, the ICUs of Okayama University Hospital admitted 3664 patients, including 728 with SIRS. About three fourth of them were one-night stay in ICU. For lack of resource availability, we were granted written consent to collect blood from 70 patients, whose characteristics are shown in Table 1 and Supplementary Table 1. The median age of patients was 67 (IQR, 62–76) years, 52 (74%) were males, and all were treated in the ICU for 6 (IQR, 4–9) days. The median patient APACHE II and SOFA scores were 15 (IQR, 12.7–18.2) and 3 (IQR, 2–5), respectively, and 20 patients (29%) were diagnosed with sepsis. ICU mortality and 90–day mortality were both 11% (eight patients) and 28–day mortality was 7.1% (five patients). We were granted written consent from 9 non-SIRS patients and 16 healthy volunteers. There were no differences in age among all groups used in analyses, except for healthy volunteers.

Plasma levels of HRG and other markers

The median HRG level in healthy volunteers (n = 16) was 63.00 (IQR, 51.53–66.21) μg/ml (Supplementary Fig. 3A). HRG levels in non-SIRS patients (n = 9, 38.64 [IQR, 30.26–51.81] μg/ml) were significantly lower than those in healthy volunteers (P = .0017). Furthermore, HRG levels in SIRS patients (n = 70, 28.72 [IQR, 15.74–41.46] μg/ml) were lower than those in non-SIRS patients (P = .049).

Supplementary Fig. 3B and Supplementary Fig. 3C show the results of secondary analyses. Comparison of patients with sepsis (n = 20) and patients with non-infective SIRS (n = 50) showed that HRG levels in the former group were significantly lower than those in the latter group (8.71 [IQR, 6.72–15.74] vs 33.27 [IQR, 26.57–44.99] μg/ml; P < .001). Moreover, PCT and presepsin levels of septic patients were significantly higher than those of non-infective SIRS patients. In SIRS patients, HRG levels of non-survivors (n = 8, 9.06 [IQR, 4.49–15.70] μg/ml) were significantly lower (P < .001) than those of survivors (n = 62, 31.78 [IQR, 18.57–42.11] μg/ml). Although presepsin levels of non-survivors (1276 [IQR, 802.7–5437] pg/ml) were significantly higher (P < .001) than those of survivors (449 [IQR, 326.7–618.7] pg/ml), their PCT levels (0.520 [IQR, 0.220–1.277] vs. 1.605 [IQR, 0.555–3.330] ng/ml) were not

significantly different (P = .73). Within septic patients, there were no differences in HRG level between survivors (n = 12) and non-survivors (n = 8) (data not shown).

Diagnostic accuracy of HRG levels

We performed ROC curve analysis to detect patients with sepsis within the group with SIRS. The ROC curve for HRG was highly sensitive and specific, with the following area under the curve (AUC) values: HRG, 0.97; PCT, 0.82; presepsin, 0.77 (Fig. 1). AUC for HRG was higher than that of PCT (p = .0018) and presepsin (p = .0012).

Associations between markers and mortality

Table 2 shows associations between the plasma level of each marker and mortality. HRG level on ICU day 1 was significantly associated with mortality (Hazard ratio [HR], 0.88; 95% confidence interval [CI], 0.80–0.98; P < .001), and when adjusted according to APACHE II score, this level remained an independent prognostic factor (adjusted HR, 0.89; 95% CI, 0.78–0.97; P = .0053). The presepsin level was significantly associated with mortality in univariate analysis (HR, 1.03; 95% CI, 1.01–1.05; P = .0040), although when adjusted using the APACHE II score, there was no significant association between presepsin level and mortality (adjusted HR, 0.99; 95% CI,

0.99–1.00; P = .49). The PCT level did not significantly associate with mortality. The Harrell C-index for mortality was as follows: HRG, 0.85; PCT, 0.65; presepsin, 0.87; APACHE II score, 0.90; SOFA score, 0.88; C-reactive protein (CRP), 0.61.

Supplementary table 2 shows that the sensitivity and specificity of HRG levels associated with mortality at the cutoff level of 16.0 μg/ml were 0.87 and 0.79, respectively. Thus, when patients were divided into higher HRG and lower HRG groups according to this cutoff level, Kaplan–Meier curves (Fig. 2) showed that the mortality of the lower HRG group was significantly higher than that of the higher HRG group (HR, 9.18; 95% CI, 1.85–45.5; P = .0028).

DISCUSSION

In this study, we found that HRG levels of SIRS patients were significantly lower than those of non-SIRS patients and that HRG levels of septic patients were lower than those of non-infective SIRS patients. In addition, HRG was significantly associated with mortality and provided sufficient diagnostic and prognostic accuracy as a biomarker for sepsis within SIRS patients.

HRG levels decreased in patients with SIRS who were treated in the ICU. To our

knowledge, there are no reports describing HRG levels in critically ill patients, although HRG levels have been shown to decrease in patients with liver insufficiency (14) and in those receiving corticosteroids (15). HRG levels have also been proposed to decrease during pregnancy and further decrease in patients with pre-eclampsia (16). In addition, HRG levels have been shown to decrease in outpatients with elevated CRP values, leading to the conclusion that HRG acts as a negative acute-phase reactant (17). In this study, we demonstrated that HRG levels decreased in patients with SIRS and were negatively correlated with CRP levels (Supplementary table 3). Our results support the conclusion that inflammation decreases HRG levels.

We evaluated HRG as a biomarker for sepsis by comparing HRG levels with levels of PCT and presepsin, both clinical biomarkers for sepsis (3). When we divided SIRS patients into groups with and without infection, ROC curve analysis for diagnosing sepsis revealed that AUC for HRG, PCT, and presepsin was 0.97, 0.82, and 0.77, respectively. These data indicate that HRG is the best marker for detecting sepsis within SIRS patients. The review article focused on the use of PCT in septic patients in an ICU setting reported that the sensitivity to detect sepsis ranged from 65 to 96 % and the specificity ranged from 70 to 89 %, which was in agreement with current study (18).

Moreover, we demonstrated that HRG and presepsin levels, but not PCT levels, were

associated with mortality. The Harrell C-index (predictive power) for mortality was 0.85 and 0.87 for HRG and presepsin, respectively, consistent with the C-index for the APACHE II score (0.90). This score is an established clinical prognostic marker used worldwide but involves a complicated scoring system calculated according to dozens of parameters. Thus, our present results strongly suggest that HRG will serve as a more effective prognostic biomarker for SIRS patients.

Using a mouse sepsis model, we clearly demonstrated that plasma HRG decreased markedly due to reduction of mRNA expression in the liver, degradation by thrombin, and deposition on intravascular thrombi (10). Under such condition, a cascade of responses including intravascular neutrophil extracellular traps (NETs) formation, strong attachment of neutrophils to vascular endothelial cells, and immunothrombus formation proceed, leading to acute respiratory distress syndrome (ARDS), disseminated intravascular coagulation (DIC), and multiple organ failure (10). Thus, the marked decrease in plasma HRG may have a direct causal relation with septic lethality.

In this study, we also developed sandwich ELISA to measure HRG levels using one mAb to capture HRG, taking advantage of the high-affinity binding of HRG to Ni–NTA (19). This method does not rely on different HRG epitopes and therefore does not require the use of multiple antibodies. Using this ELISA, we determined that the median

HRG level in healthy volunteers was 63.00 (IQR, 51.53–66.21) μg/ml, in agreement with published data showing that HRG levels are approximately 100 μg/ml in human plasma and that they vary widely (6, 5). We therefore conclude that HRG ELISA developed in this study is acceptable for clinical practice.

There are limitations to this study. First, our study was a single-center study and included only 79 patients. However, to address this limitation, we initiated a multicenter prospective study to validate the diagnostic and prognostic role of HRG levels. Second, although we focused on sepsis, we studied 70 patients with SIRS, including only 20 who had the disease. Thus, it is unclear whether HRG would work to differentiate sepsis survival due to limited numbers of individual analyzed here. Larger validation studies focused on sepsis should be performed. Third, in this study, we used old definition of sepsis (Sepsis-2) because we conducted this study from November 2012 through November 2014. We should initiate another study with new definition for sepsis (Sepsis-3) (20). Fourth, we only assessed the initial HRG level and we had no serial data. Time-dependent changes in HRG levels would be more valuable and reflect treatments such as steroids and renal replacement therapies. Further investigations about serial values would be needed. Fifth, non-infective SIRS patients included many postoperative patients. Postoperative condition is complicated because of the effects of

general anesthesia, surgical pain, or something associated with operation. These effects might confuse a comparison between non-infective SIRS patients and septic patients. Sixth, non-infective SIRS patients included many patients with cancer. Because HRG levels may prevent tumor growth (21, 22, 5) and are significantly higher in patients with breast cancer (23), the characteristics of these populations may have influenced our data.

CONCLUSIONS

HRG levels of septic patients were significantly lower than those of non-infective SIRS patients and HRG levels were significantly associated with mortality within the SIRS population. Therefore, HRG may be superior to PCT and presepsin for assessing severity of SIRS patients. Our results suggest that HRG serves as a novel biomarker for diagnosing sepsis, evaluating severity of patients, and predicting patient outcomes. To confirm our findings, larger validation studies are needed.

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FIGURE LEGENDS

Fig 1. Receiver-operating characteristic (ROC) curve analysis for detecting sepsis

ROC curves of HRG, PCT, and presepsin. The area under the curve (AUC) in ROC curve analysis for HRG was 0.97. AUC for HRG was higher than that of PCT (0.82, $p = 0.0018$) and presepsin (0.77, $p = 0.0012$).

Fig 2. Kaplan-Meier survival curves

Patients were divided into higher HRG and lower HRG groups according to the cutoff level of 16.0 μg/ml. At the cutoff level of 16.0 μg/ml, the sensitivity and specificity of HRG levels associated with mortality were 0.87 and 0.79, respectively.

Supplementary Fig 1. Linearity of enzyme-linked immunosorbent assay (ELISA)

Typical data of standard curve were shown.

Supplementary Fig 2. SDS-PAGE and western blotting pattern of purified human HRG

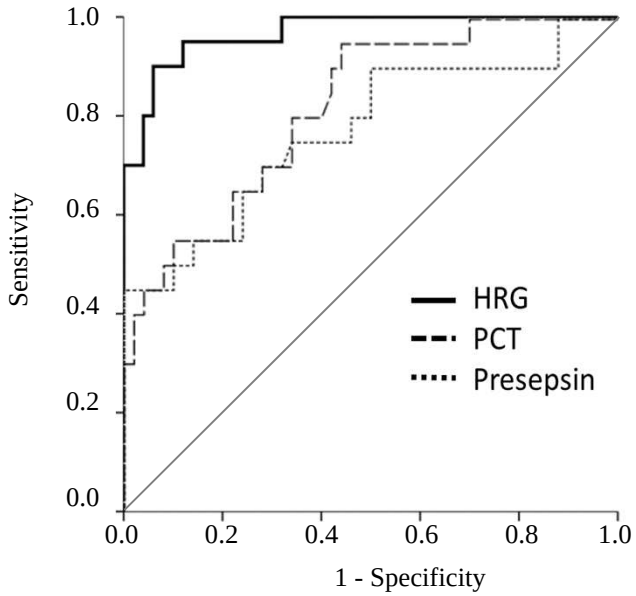
Purified HRG was electrophoresed and the gels were stained by Coomassie Brilliant

Blue (CBB). HRG band was detected by western blotting method with rabbit anti-human HRG polyclonal antibody (made in-house). CBB denote Coomassie Brilliant Blue. WB denote Western Blotting.

Supplementary Fig 3. Plasma levels of each marker

(A) Plasma levels of HRG. We compared 4 groups of healthy volunteers, non-SIRS, non-infective SIRS, and sepsis. There were significant differences between groups of non-SIRS and sepsis, and non-infective SIRS and sepsis. (B) Plasma levels for each marker used to compare patients with and without infection (sepsis and non-infective SIRS) within SIRS population. (C) Plasma levels for each marker used to compare survivors and non-survivors within SIRS population. A box-and-whisker plot showing median, 25^{th}, and 75^{th} percentiles. The bars represent the 5^{th} and 95^{th} percentiles. A triangle and open square show outlier. * $P < .05$. *** $P < .001$.

Figure 1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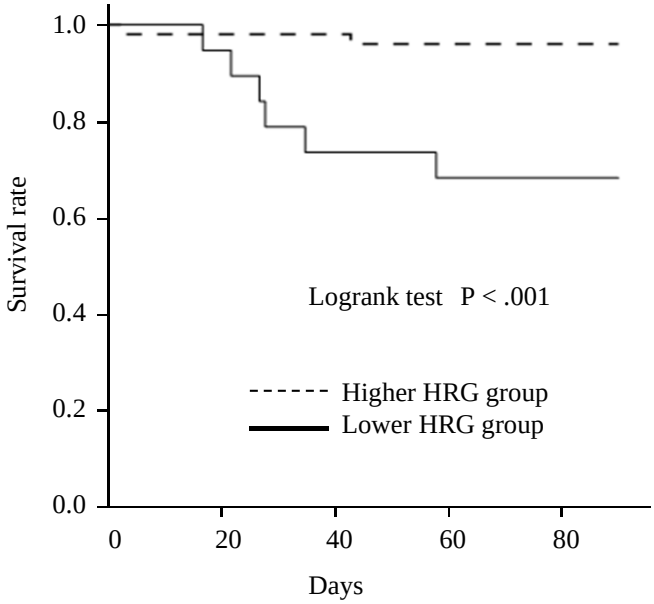
HRG
PCT
Presepsin

Figure 2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Lower HRG group
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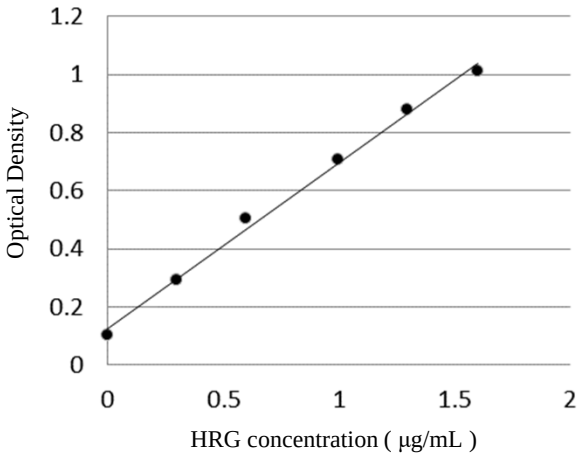
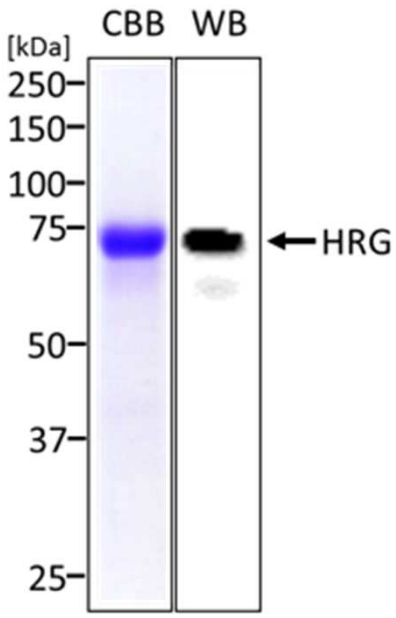
Supplementary Figure 1



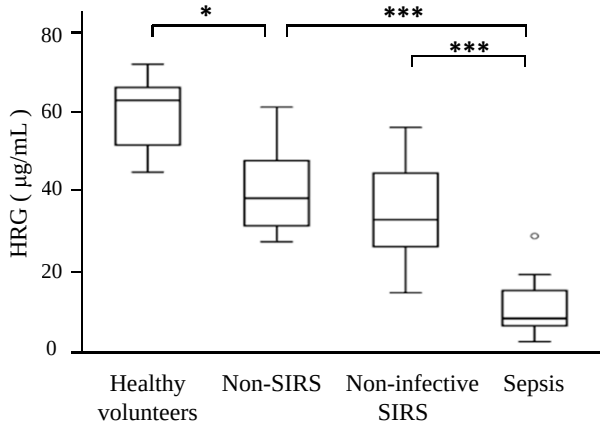
(μg/mL)	O.D.	Average
0	0.102 0.125 0.074	0.100
0.3	0.319 0.302 0.260	0.294
0.6	0.517 0.522 0.469	0.503
1.0	0.737 0.718 0.669	0.708
1.3	0.887 0.932 0.819	0.879
1.6	1.030 1.053 0.948	1.010

Supplementary Figure 2



Supplemental Figure 3

(A)



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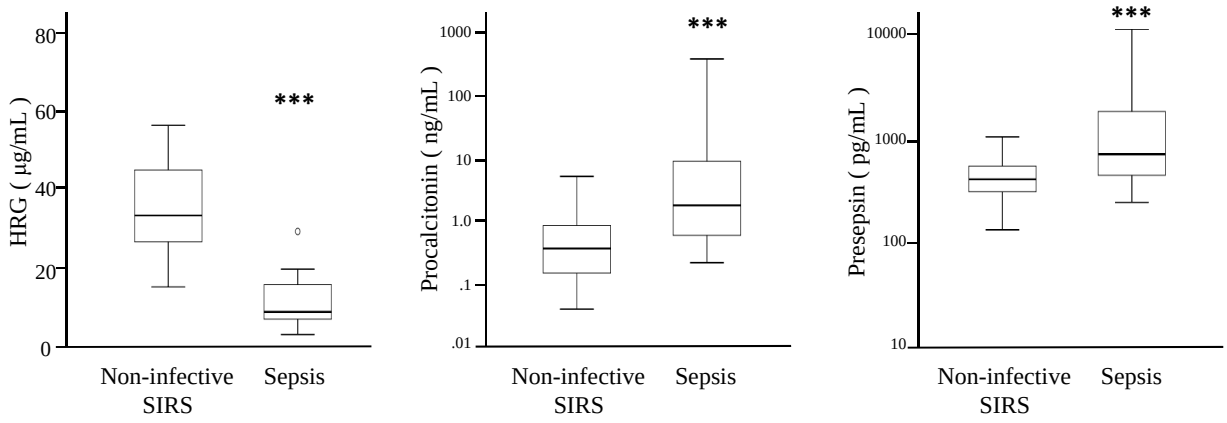

(C)

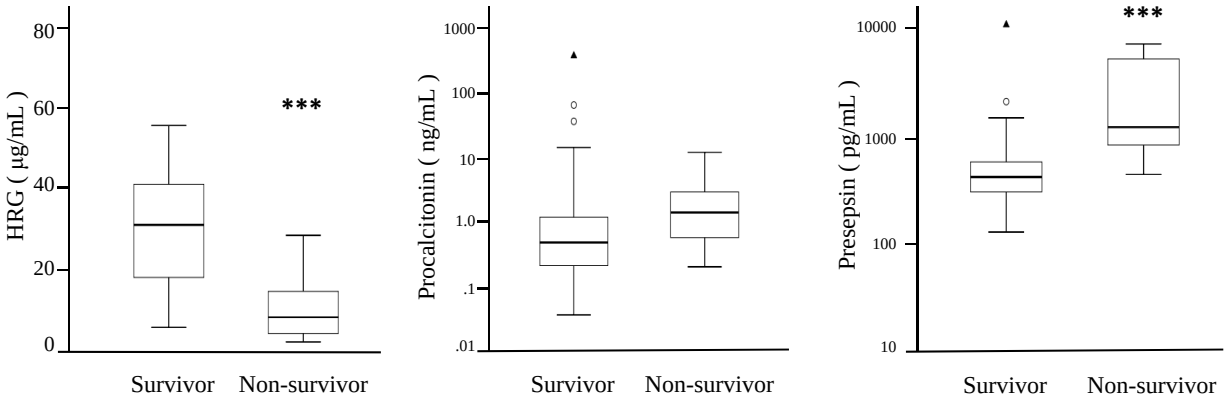

Table 1. Patient characteristics

Expressed as median (IQR).

Variable	Healthy volunteers	Non-SIRS patients	SIRS Patients		
			Total	Non-infective SIRS	Sepsis
N	16	9	70	50	20
Age, years	31.5 (25.7-35.7)	68.0 (66.0-72.5)	67 (62-76)	66.5 (62.7-74.5)	68 (60.5-77)
Male sex	12 (75%)	6 (66.7%)	52 (74.2%)	39 (78%)	13 (65%)
ICU death		0	8 (11%)	0	8 (40%)
28-day death		0	5 (7.1%)	0	5 (25%)
90-day death		0	8 (11%)	0	8 (40%)
ICU stay, days		5 (3.5-6.5)	6 (4-9)	6 (4-7.2)	14 (6.25-26.5)
Severity of disease					
APACHE II score		13.0 (10.5-15.5)	15 (12.7-18.2)	14 (12-16)	19.5 (17.2-28.5)
SOFA score		1 (0-2)	3 (2-5)	2 (2-4)	8 (5.2-12)
Severe Sepsis		0	9 (13%)	0	9 (45%)
Septic Shock		0	8 (11%)	0	8 (40%)
Medical patients		0	16 (23%)	1 (2%)	15 (75%)
Pneumonia		0	6	0	6
Renal failure		0	3	0	3
Hepatic failure		0	1	0	1
Pancreatitis		0	1	0	1
Brain infarction		0	1	1	0
Ileus		0	1	0	1
Others		0	3	0	3
Surgical patients		9	54 (77%)	49 (98%)	5 (25%)
Abdominal		1	8	3	5
Esophageal		4	21	21	0
Laryngeal		3	18	18	0
Hepatic		0	2	2	0
Others		1	5	5	0
Ventilation days		1 (1-1)	1 (0-1)	1 (1-1)	0 (0-7.5)
Vasopressors		0	10 (14%)	2 (4.0%)	8 (40%)
Blood Purification		0	7 (10%)	0	7 (35%)
Corticosteroids		0	9 (13%)	1 (2.0%)	8 (40%)

Table 2. Associations between each marker and mortality

HR denotes hazard ratio. Adjusted HR denotes hazard ratio adjusted according to Acute Physiology and Chronic Evaluation (APACHE) II score.

Variable	Univariate analysis			Adjusted with APACHE II score	
	HR (95%C.I.)	P	Harrell C-index	Adjusted HR (95%C.I.)	P
HRG	0.88 (0.80 to 0.98)	< .001	0.85	0.89 (0.78 to 0.97)	.0053
PCT	0.97 (0.84 to 1.13)	.76	0.65		
Presepsin	1.03 (1.01 to 1.05)	.0040	0.87	0.99 (0.99 to 1.00)	.49

Supplementary Table 1. Patient characteristics

Expressed as median (IQR).

Variable	SIRS Patients			
	Total N=70	Non-infective SIRS N=50	Sepsis N=20	P
Biochemical data (ICU Day 1)				
WBC (/L)	8380 (5455-12280)	8380 (5500-11460)	8430 (2680-14610)	.78
Ht (%)	29.6 (27.0-33.4)	30.3 (27.5-33.5)	28.5 (23.7-33.1)	.024
Plt (*10000/L)	15.9 (10.2-22.5)	17.3 (12.9-23.0)	8.65 (5.10-15.2)	< .001
CRP (mg/dL)	6.46 (4.38-11.5)	5.94 (4.28-7.65)	17.1 (9.59-21.3)	< .001
BUN (mg/dL)	14.7 (10.8-18.8)	12.7 (10.3-16.1)	33 (13.6-38.4)	< .001
Cr (mg/dL)	0.78 (0.59-1.04)	0.73 (0.58-0.90)	1.27 (0.73-2.73)	< .001
Bil (mg/dL)	0.89 (0.56-1.33)	0.82 (0.54-1.15)	1.37 (0.61-2.12)	.0029
AST (U/L)	32.0 (20.0-61.5)	31.5 (21.7-60.0)	41.0 (16.7-61.7)	.18
Total Protein (g/dL)	4.5 (4.1-4.8)	4.4 (4.0-4.6)	4.7 (4.1-5.6)	.0059
ALB (g/dL)	2.5 (2.1-2.8)	2.5 (2.1-2.8)	2.5 (2.1-2.8)	.67
Lac (mmol/L)	2.1 (1.2-2.9)	2.1 (1.7-2.7)	2.0 (1.6-5.2)	.0055
Fbg (mg/dL)	450 (308-525)	437 (297-491)	525 (367-582)	.010

Supplementary Table 2. Significance of HRG for predicting mortality

PPV and NPM denote positive and negative predictive values, respectively.

Cut off values (μg/mL)	Sensitivity	Specificity	PPV	NPV
5.0	0.38	1.00	1.00	0.91
10.0	0.50	0.87	0.33	0.93
15.0	0.75	0.85	0.40	0.96
16.0	0.88	0.79	0.35	0.98
20.0	0.88	0.71	0.28	0.98
25.0	0.88	0.68	0.30	0.98
30.0	1.00	0.52	0.21	1.00

Supplementary Table 3. Correlations between HRG and other parameters

ρ denote Spearman's rank correlation coefficient.

Variable	ρ	P
WBC	0.025	0.84
CRP	–0.46	<.001
PCT	–0.56	0.22
Presepsin	–0.33	0.0060
SOFA score	–0.51	<.001
APACHE II score	–0.38	.0010