

## Serum Hepcidin-25 Levels Reflect the Presence of Bacteremia in Patients with Systemic Inflammatory Response Syndrome

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**Background:** Hepcidin-25 is a key regulatory hormone of iron homeostasis in humans, and its production is greatly upregulated by inflammation as well as iron overload. The aim of this study was to investigate the pathophysiological role of hepcidin-25 in patients with systemic inflammatory response syndrome (SIRS).

**Methods:** We enrolled 113 consecutive patients (aged 63.4±21, 50 men, 63 women), with 2 or more SIRS criteria, who were admitted to our department of general medicine between August 1, 2015 and August 31, 2017. We measured complete blood cell count and serum levels of hepcidin-25, iron, iron-binding capacity, ferritin, blood urea nitrogen, creatinine, albumin, and C-reactive protein (CRP) on admission. The patients were divided into 3 groups: a bacteremia group (27 patients), a culture-negative bacterial infection group (60 patients), and a non-bacterial infection group (26 patients).

**Results:** Hepcidin-25 levels were found to be comparable in terms of SIRS criteria: 162 [2.8–579], 193 [2.24–409], and 180 [89.2–421] ng/mL in patients with 2, 3, and 4 criteria, respectively ( $P=0.533$ ). However, hepcidin-25 levels were significantly higher in the bacteremia group (209 [56.7–579] ng/mL) than in either the culture-negative bacterial infection group (168 [2.24–418] ng/mL) or the non-bacterial infection group (142 [2.8–409] ng/mL). A significant positive correlation between hepcidin-25 and CRP levels was noted in the bacteremia group ( $r=0.528$ ,  $P=0.005$ ) and non-bacterial infection group ( $r=0.648$ ,  $P<0.001$ ). Moreover, iron and ferritin levels were significantly lower in the bacteremia group than in the non-bacterial infection group.

**Conclusions:** Our findings suggest that hepcidin-25 level may reflect the presence of bacteremia as well as the severity of inflammation in patients with SIRS. (J Nippon Med Sch 2019; 86: 91–97)

**Key words:** hepcidin-25, bacteremia, SIRS

### Introduction

Hepcidin is a 25-amino acid peptide hormone and plays a significant role in iron homeostasis in humans<sup>1</sup>. The bioactive hepcidin-25 is synthesized predominantly in the liver and its N-terminal processing results in smaller isoforms (hepcidin-24, -23, -22, and -20) with greatly reduced activity that accumulate in the urine<sup>2–4</sup>. It induces internalization and degradation of the cellular iron exporter, ferroportin, on enterocytes, macrophages, and hepatocytes<sup>5,6</sup>. Therefore, increased hepcidin-25 production leads to a decrease in serum iron concentrations and an increase in iron storage as ferritin<sup>7</sup>. Recent studies have

suggested that hepcidin-25 plays a key role in host-pathogen interface. Iron is essential for the survival and growth of bacteria. When the bacteria infect macrophages, macrophages release a number of cytokines, including IL-6, which increase production of hepcidin-25 in hepatocytes. This up-regulation of hepcidin-25 leads to iron depletion and inhibition of bacterial growth<sup>8,9</sup>.

Systemic inflammatory response syndrome (SIRS) may occur in several conditions related to bacterial infection, viral infection, autoimmune disorders, and malignancy. In humans, increased serum hepcidin-25 levels were noted in patients with chronic infections and severe in-

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flammatory diseases where inflammatory cytokines play a pivotal role<sup>10</sup>. Inflammation-induced hepcidin-25 was also demonstrated and associated with the development of anemia in septic patients<sup>11</sup>. Although hepcidin-25 appears to be involved in such various inflammatory diseases and is considered as a component of innate immunity, there are no clinical studies that have comprehensively examined an association between hepcidin-25 levels and the severity of inflammation that occurs from miscellaneous causes, regardless of bacterial infection. Therefore, the aim of this study was to elucidate the clinical importance of serum hepcidin-25 levels in patients with SIRS.

## Materials and Methods

### Patients and Sampling

This observational study was carried out between August 1, 2015 and August 31, 2017. The Ethics Committee of Nippon Medical School approved the research protocol, confirming that the study was planned in accordance with the Declaration of Helsinki. We enrolled 113 consecutive patients (aged 63.4±21, 50 men, 63 women) who suffered from inflammatory diseases and were admitted to the department of General Medicine with 2 or more of the SIRS criteria: temperature >38°C or <36°C, heart rate >90/min, respiratory rate >20/min or PaCO<sub>2</sub> <32 mmHg, and white blood cell count (WBC) >12,000/mm<sup>3</sup> or <4,000/mm<sup>3</sup> or >10% immature bands<sup>12</sup>. The assessment of SIRS was performed just before their hospitalization. Informed consent from patients or relatives was obtained in advance. Blood samples were collected at day 1, 2, and 3 after admission for measurement of the following parameters: complete blood cell count with differential and serum levels of blood urea nitrogen, creatinine, albumin, C-reactive protein (CRP), hepcidin-25, ferritin, and iron. All parameters except for hepcidin-25 were measured by a hematology autoanalyzer (Sysmex, Japan) or an automated biochemical analyser (Hitachi High-Technologies, Japan) on the day of sampling. For serum hepcidin-25 isoform measurement, blood samples collected at each sampling point were centrifuged to obtain serum samples, which were frozen and stored at -80°C for 3 to 4 months before analysis. The hepcidin-25 concentration was determined using a specific ELISA kit (Peninsula Laboratories, LLC, A Member of the Bechem Group, 305 Old Country Rd., San Carlos, CA 94070, USA). The normal range of the serum hepcidin-25 in healthy volunteers was 1.6–12.7 ng/mL<sup>13</sup>.

The patients were divided into 3 groups for compar-

son: 27 patients having bacterial infection with positive results of blood culture (bacteremia group), 60 patients having bacterial infection with negative results (culture-negative group), and 26 patients with SIRS except for bacterial infection (non-bacterial infection group). Two or more sets of blood cultures were obtained from all patients unless bacterial infection was obviously excludable. If only a single blood culture grew an organism known to often cause contamination, the case was classified into the culture-negative bacterial infection group. The diagnosis of bacterial infection was based on the combination of culture results for blood, urine or sputum culture with imaging modalities including chest X-ray and CT scan of the chest or abdomen. The clinical spectrum of bacterial infection included lower respiratory tract infections, urinary tract infections, and intra-abdominal infections. The diagnosis of viral infection was made by use of an IgM antibody specific for a viral agent. The non-bacterial infection group consisted of 11 patients with viral infection, 7 with autoimmune diseases, 3 with malignant diseases, 3 patients with trauma, one with heat stroke, and one with TAFRO syndrome<sup>14</sup>.

### Statistical Analysis

All statistical analyses and data management were performed using IBM SPSS Statistics, Version 24 for Windows. Continuous variables with normal distribution were presented as mean±standard deviations, and non-normally distributed variables were expressed as median and range (minimum–maximum). The Kruskal-Wallis test was used to compare medians among the 3 groups. If statistical significance was achieved, intergroup comparisons were made with the Mann-Whitney U test with Bonferroni correction. Age was compared using the Analysis of Variance among the 3 groups. The frequencies of categorical variables were compared using Pearson's chi-square test, when appropriate. The relationship between hepcidin-25 levels with other variables was examined using the Spearman correlation coefficient. A value of P<0.05 was considered significant.

## Results

### Demographic Information, Laboratory Data, and Blood Culture Results

Demographic information and laboratory results of each patient group are shown in **Table 1**. Although the patients in the non-bacterial infection group appeared to be younger than those of either the bacteremia- or culture-negative group, that was not statistically significant. Three patients died during hospitalization: two

Table 1 Demographic information and laboratory data in each group

	Bacteremia N=27	Culture-negative N=60	Non-bacterial infection N=26	P-value
Age (years)	68±18	65±21	58±24	0.195
Gender, male (%)	37	45	50	0.628
In-hospital death (%)	7.4	0	3.8	0.126
WBC (/μL)	14,100 (1,300–29,000)	11,950 (4,900–51,900)	12,150 (30,200)	0.322
Neutrophils (%)	89.4 (63.2–95.6)	86.5 (61.3–98.2)	72.9 (14–92.7)*	<0.001
Hemoglobin (g/dL)	12.5 (8.1–15.2)	12.6 (7.7–17)	11.7 (5.3–18)	0.555
Blood urea nitrogen (mg/dL)	21.8 (5.1–75.7)*	17.1 (4.8–73.2)	12.3 (5.2–53.1)	0.003
Creatinine (mg/dL)	1.09 (0.63–2.92)*	0.81 (0.47–4.36)	0.73 (0.38–1.71)	0.001
Albumin (g/dL)	3.4 (2–4.4)	3.5 (1.9–4.7)	3.4 (1.7–4.4)	0.781
C-reactive protein (mg/dL)	17.24 (0.21–33.45)*	10.12 (0.12–35.02)	5.66 (0.04–47.8)	0.003
Iron (μg/dL)	13 (5–77)*	19 (6–92)	27 (9–258)	0.01
UIBC (μg/dL)	239 (146–396)	228 (97–399)	177 (20–352)	0.109
Ferritin (ng/mL)	271 (48.5–704.7)	253 (10.3–1,791)	678 (15.2–4,382)*	0.003
Day 1 Hepcidin-25 (ng/mL)	209 (56.7–579)*	168 (2.24–418)	142 (2.8–409)	0.01
Max. Hepcidin-25 (ng/mL)	256 (56.7–579)	192 (17.7–418)	145 (4–471)	0.041

Data are expressed as mean±standard deviation, percentage of total, or median (minimum to maximum).

\*P<0.05 vs the other groups.

Table 2 Blood culture results in the bacteremia group

Pathogens	Number of cases
<i>Escherichia coli</i>	13
<i>β-streptococcus</i>	5
<i>Klebsiella pneumoniae</i>	2*
<i>Enterococcus faecalis</i>	2*
<i>Proteus mirabilis</i>	1
<i>Staphylococcus aureus</i>	1
MRSA	1
<i>Streptococcus anginosus</i>	1
<i>Enterobacter cloacae</i>	1
GNR (unable to grow and undetected)	1

\*One case revealed co-infection with *Klebsiella pneumoniae* and *Enterococcus faecalis*.

from sepsis (bacteremia group) and one from intravascular lymphoma (non-bacterial infection group). There were also no significant differences among the 3 groups in gender, WBC, hemoglobin concentrations, and serum levels of albumin and UIBC. However, values for neutrophil differential, blood urea nitrogen, creatinine, and CRP were significantly higher in the bacteremia group than in the other groups. Instead, both iron and ferritin levels were significantly lower in the bacteremia group than in the other groups. Blood culture results are presented in **Table 2**. The major pathogen identified by blood culture was *Escherichia coli*, followed by *β-Streptococcus*. One case was positive for both *Klebsiella pneumoniae* and *Enterococcus faecalis*, indicating a co-infection.

### Hepcidin-25 Levels and SIRS Scores

Hepcidin-25 levels on day 1 were 162 [2.8–579] ng/mL in patients with 2 criteria (n=61), 193 [2.24–409] ng/mL in those with 3 (n=39), and 180 [89.2–421] ng/mL in those with 4 (n=13), respectively (P=0.533) (**Fig. 1**). There was no association between hepcidin-25 levels and SIRS scores.

### Hepcidin-25 Levels with or without Bacteremia

The hepcidin-25 levels on day 1 were significantly higher in the bacteremia group (209 [56.7–579] ng/mL) than in either the culture-negative group (168 [2.24–418] ng/mL) or the non-bacterial infection group (142 [2.8–409] ng/mL; P<0.05) (**Fig. 2**). Nevertheless, there was no significant difference in the maximum hepcidin-25 levels among the 3 groups.

### Correlation between Hepcidin-25 Levels and Other Parameters

The correlations between the serum hepcidin-25 levels on day 1 and parameters of inflammation and ferrokinetics are shown in **Table 3** and **Figure 3**. In the bacteremia group, a positive correlation was present between hepcidin-25 and CRP (r=0.528, P=0.005), but there was no significant correlation between hepcidin and the other parameters. In the non-bacterial infection group; hepcidin-25 had a positive correlation with WBC (r=0.466, P=0.016), CRP (r=0.648, P<0.001), and ferritin (r=0.529, P=0.005). It also had a negative correlation with iron (r=-0.479, P=0.013) and UIBC (r=-0.391, P=0.048). In the culture-negative bacterial infection group, hepcidin appeared to have a positive correlation with ferritin (r=

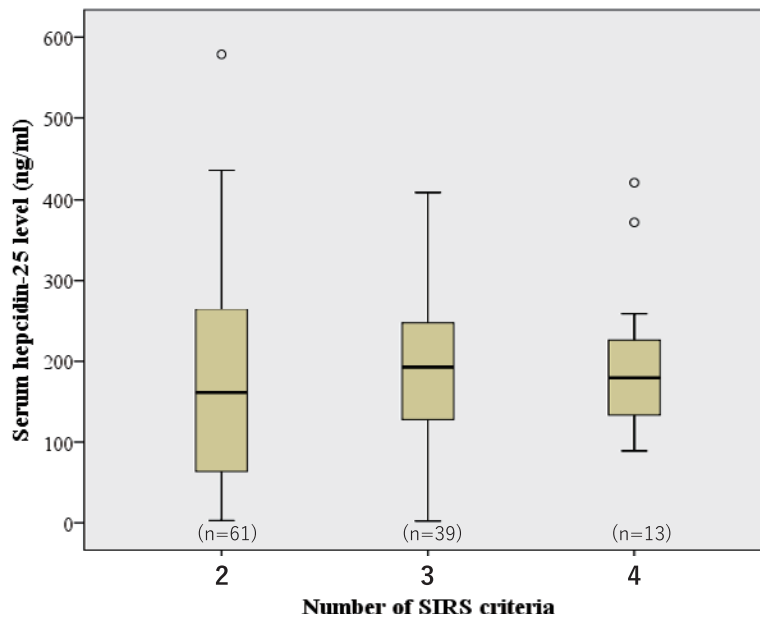


Fig. 1 Box plots of serum hepcidin-25 levels in order of the numbers of systemic inflammatory response syndrome (SIRS) criteria. Center bars indicate median values. Box edges show the 25th and 75th percentiles with whiskers to the 1.5 interquartile range. Open circles represent outliers.

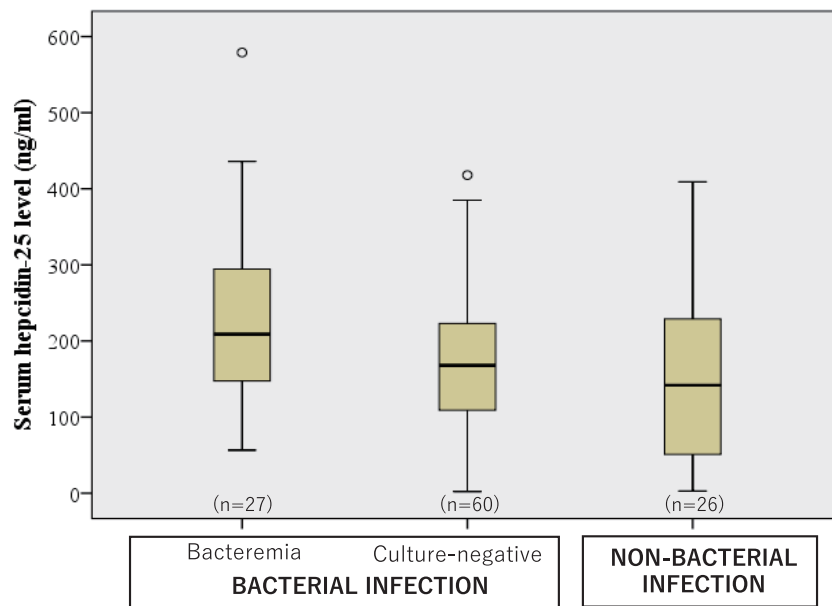


Fig. 2 Box plots of serum hepcidin-25 levels of the bacteremia group, the culture-negative group, and the non-bacterial infection group. Center bars indicate median values. Box edges show the 25th and 75th percentiles with whiskers to the 1.5 interquartile range. Open circles represent outliers.

0.435,  $P=0.001$ ) and a weak negative correlation with UIBC ( $r=-0.289$ ,  $P=0.025$ ), but there was no correlation between hepcidin and CRP or WBC.

### Discussion

The present study is the first to examine the pathophysi-

ologic relevance of hepcidin-25 in patients with SIRS from various causes, including bacterial/viral infection and non-infectious diseases. Although hepcidin-25 levels were not associated with the number of SIRS criteria present, they were significantly higher in patients with bacteremia than in those without.

Table 3 Correlation between serum hepcidin-25 levels and other laboratory parameters in each group

	Bacteremia		Culture-negative		Non-bacterial infection	
	r	P	r	P	r	P
WBC (/μL)	-0.020	0.922	0.109	0.407	0.466*	0.016
Neutrophils (%)	0.220	0.292	0.012	0.929	0.253	0.222
C-reactive protein (mg/dL)	0.528*	0.005	0.141	0.284	0.648*	<0.001
Iron (μg/dL)	-0.161	0.423	0.114	0.387	-0.479*	0.013
UIBC (μg/dL)	-0.231	0.247	-0.289*	0.025	-0.391*	0.048
Ferritin (ng/mL)	0.323	0.100	0.435*	0.001	0.529*	0.005

\*P<0.05

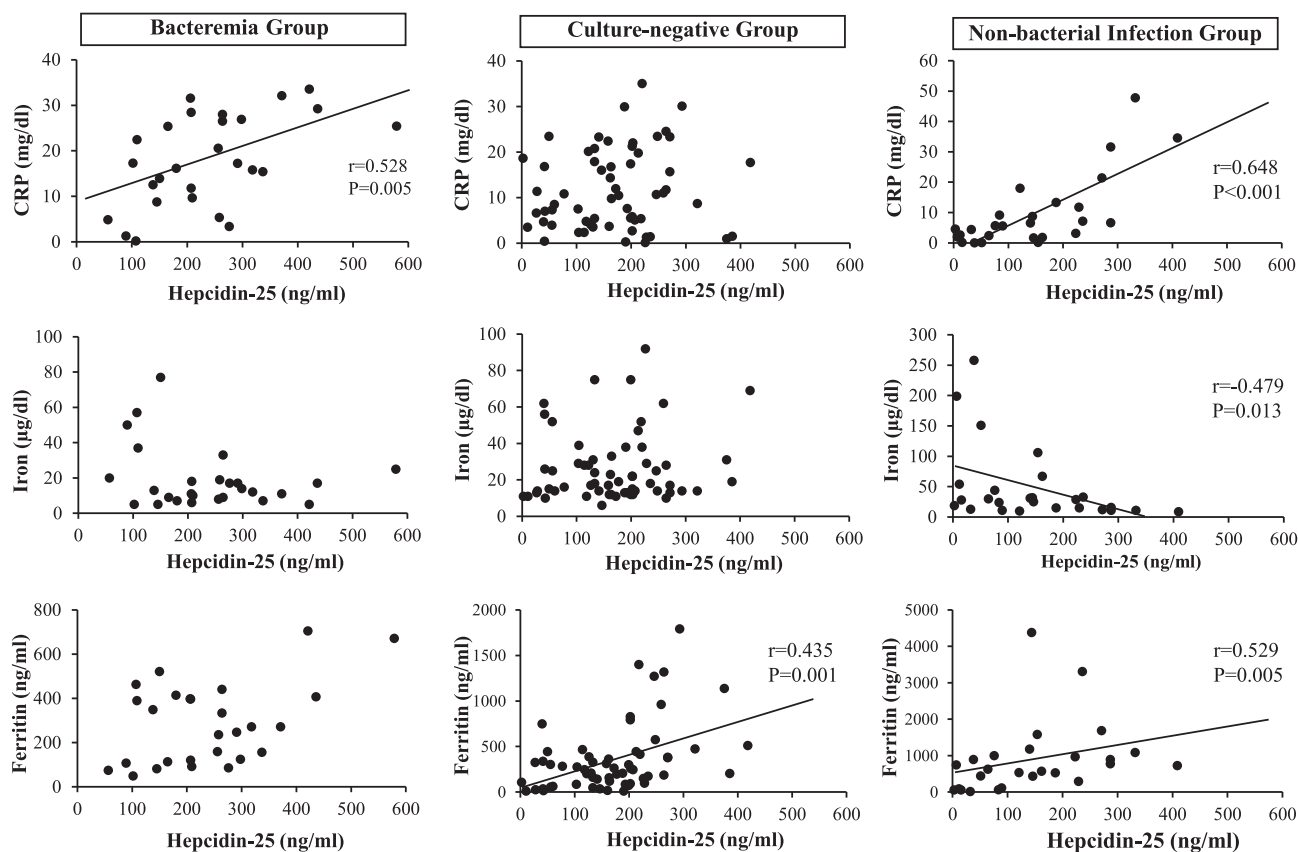


Fig. 3 Scattergrams showing correlations of CRP, iron and ferritin against hepcidin-25 in each study group. The left column shows the bacteremia group, the middle column shows the culture-negative group, and the right column shows the non-bacterial infection group. The linear regression lines are drawn if a significant correlation is indicated.

As noted earlier, hepcidin-25 may be involved in various inflammatory diseases. In animal models, infections or stimuli that invoke a systemic inflammatory response are likely to induce liver hepcidin expression, reduce serum iron, and increase iron accumulation as ferritin in reticuloendothelial cells<sup>15</sup>. Thus, it is reasonable to assume that the extent of hepcidin upregulation and resultant hypoferrremia is greater if the inflammatory response becomes more vigorous. However, only a limited number of studies have tested this hypothesis in humans. An observational study in septic patients suggested that hep-

cidin levels correlated with the number of extended SIRS criteria and levels of IL-6 and that hepcidin was associated with development of anemia<sup>11</sup>. Our study failed to show a significant relationship between hepcidin-25 levels and the number of SIRS criteria. This discrepancy could be explained by the difference in patient selection and definition of SIRS: they analysed septic patients alone using the extended SIRS criteria, whereas we applied the classical criteria to the patients with various causes, including non-bacterial infection. Although SIRS criteria have been proposed as a useful index of severity

for critically ill patients<sup>16</sup>, it does not necessarily mean that they are generally proportional to the extent of inflammation. Nevertheless, it should be noted that there was a positive correlation between hepcidin-25 and CRP levels in the bacteremia group and non-bacterial infection group, which is consistent with the idea of inflammation-induced hepcidin upregulation. Moreover, iron concentrations were significantly lower in patients with bacteremia than in those without, which may support the iron-sequestration strategies as a host-defence against bacteria. Similar findings have been observed by others in very low birthweight infants with or without late-onset sepsis<sup>17</sup>. They also found a positive correlation between hepcidin and CRP, and a significant stepwise increment in mean hepcidin concentration: from infants without sepsis, to septic patients with negative blood cultures, to those with positive blood cultures.

The non-bacterial infection group in our study included 26 patients with SIRS from miscellaneous causes other than bacterial infection, including viral infection, autoimmune diseases, malignant diseases, trauma, heat stroke, and TAFRO syndrome. Surprisingly, the correlation coefficient between hepcidin-25 and CRP is higher in the non-bacterial infection group than in the bacteremia group ( $r=0.648$  vs  $0.528$ ). In addition, serum hepcidin-25 in the non-bacterial infection group was negatively correlated with serum iron ( $r=-0.479$ ,  $P=0.013$ ) and positively correlated with ferritin ( $r=0.529$ ,  $P=0.005$ ), which was not observed in the bacteremia group. It is natural to assume that hepcidin-mediated ferrokinetic changes may reflect the time course of inflammation. In the very early stage of inflammation, the mechanism of iron sequestration would not be fully operational, particularly in some cases of acute bacterial infection. Furthermore, some of the non-infectious diseases, including collagen diseases, malignant lymphoma, or TAFRO syndrome, are supposed to stand for rather chronic inflammatory processes. This may explain why the ferritin levels were highest in the non-bacterial infection group. It should be stressed that hepcidin upregulation and iron depression take place even in the absence of bacterial infection as long as a systemic inflammatory response is triggered.

One would argue that determination of hepcidin-25 levels in patients with SIRS may be helpful to predict bacteremia or sepsis. Indeed, in the study of very low birthweight infants<sup>17</sup>, the diagnostic ability of serum hepcidin as a biomarker for late-onset sepsis has been discussed. They have shown that, in the relatively homogeneous patient groups with suspected bacteremia, serum

hepcidin is better than CRP in predicting neonatal sepsis using Receiver Operating Characteristic (ROC) curve analysis. In contrast, our patient group was substantially heterogeneous in terms of age and background disorders because the patients were consecutively registered solely by SIRS criteria. As a result, hepcidin-25 values varied considerably depending on the degree of inflammation, regardless of the presence or absence of bacterial infection. In fact, the highest value of hepcidin in the non-bacterial infection group was detected in the patient with TAFRO syndrome (409 ng/mL), a lymphoproliferative disorder characterized by overproduction of IL-6<sup>18</sup>. If the ROC curve analysis is applied to our patients with bacterial infection in order to identify the culture-positive group, the area under the ROC curve is 0.674. That implies that hepcidin-25 on its own might not be a feasible diagnostic biomarker as expected. Another problem is the accessibility for hepcidin-25 measurement because it takes 2 days due to manual measurement.

This study has some unavoidable limitations. First, we were unable to strictly distinguish patients with prehospital treatments (e.g. physical cooling, antipyretic/antimicrobial agents) from those without, which might have affected the SIRS scores on admission and the result of blood cultures. Second, this was a single-center study, and its sample size was relatively small. Thus, further study with a larger sample size is needed to confirm the results. Thirdly, because we considered that CRP could be a reasonable surrogate marker for inflammation, we did not measure IL-6, a key mediator necessary for hepcidin-25 production. Although IL-6 is thought to be the main mediator of CRP production, other cytokines such as tumor necrosis factor, IL-1, and transforming growth factor- $\alpha$  are also involved. Therefore, the results should be interpreted with caution.

As far as our best knowledge from the literature, there has been no previous study to look into hepcidin-25 levels in patients with SIRS, including non-infectious diseases. As expected from the iron-infection axis, hepcidin-25 seems to play a substantial role in host-defence, especially in patients with bacteremia or sepsis. Our findings also suggest that upregulation of hepcidin-25 should occur even in patients without bacterial infection in proportion to the degree of the systemic inflammatory response. Further studies need to be carried out to investigate the pathophysiologic role of hepcidin-25 in SIRS, especially from non-infectious causes.

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**Conflict of Interest:** The authors declare no conflicts of interest.

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