

# 学位論文

Exploration of autonomic regulation reflecting on pathophysiological change of sepsis:  
a prospective observational study

(敗血症の病態生理学的変化に対する自律神経応答の探索: 前向き観察研究)

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## Original Article

## Exploration of autonomic regulation reflecting on pathophysiological change of sepsis: a prospective observational study

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**Aim:** It remains unclear how autonomic regulation modulates pathophysiological changes of sepsis. This study aims to analyze and clarify those in patients with suspected sepsis.

**Methods:** In this single-centered, prospective, observational study, adult patients who had an infection, a quick Sequential Organ Failure Assessment score of 2 or more at the emergency department, and underwent intensive care were screened. Heart rate variability (HRV) and serum adrenaline were measured immediately after arrival. The primary outcome was defined as vasopressor dependence during 48 h after arrival.

**Results:** A total of 63 patients were included. All the patients had SOFA score of 2 or more on admission. Vasopressor dependence, renal replacement therapy, and in-hospital mortality were associated with higher adrenaline (which reflects sympathetic adrenergic system activity). Bacteremia was associated with lower high-frequency components of HRV (parasympathetic nerve activity). The HRV parameter of sympathetic nerve activity had no significant association with the outcomes. In the multivariate logistic regression model adjusted for age and sex, vasopressor dependence remained associated with higher adrenaline (cut-off 0.11 ng/mL, odds ratio 9.71, 95% confidence interval 2.55–37;  $P = 0.000874$ ), and lower high-frequency components with bacteremia (17.2 ms<sup>2</sup>, odds ratio 4.86, 95% confidence interval 1.36–17.4;  $P = 0.0152$ ). There were no significant correlations between parameters of HRV and serum adrenaline.

**Conclusion:** Hypoperfusion, organ dysfunction, and in-hospital mortality were associated with an increased sympathetic adrenergic activity. Bacteremia was associated with decreased parasympathetic nerve activity. The autonomic regulator may involve a multilayered and differentiated modulating process for sepsis.

**Key words:** Adrenaline, autonomic nervous system, bacteremia, sepsis, septic shock

## INTRODUCTION

RECENTLY, AUTONOMIC REGULATOR associated with sepsis has become a significant issue for

researchers.<sup>1,2</sup> This includes the sympathetic nervous system (SNS), parasympathetic nervous system (PNS), sympathetic adrenergic system (SAS), arginine vasopressin, hypothalamic–pituitary–adrenocortical axis, and renin–angiotensin–aldosterone system,<sup>3</sup> and is thought to respond differently to various types and intensity of the stressor.<sup>4</sup>

Heart rate variability (HRV), derived from the fluctuation of R–R intervals on original data of electrocardiogram (ECG), is a major method for evaluating SNS and PNS activities (Fig. S1). However, previous studies on HRV with the prediction outcome of sepsis are conflicting<sup>5–7</sup> because (i) the specific clinical features reflected by HRV were unclear (e.g., inflammation, hypoperfusion, and organ dysfunction), and (ii) prior studies included the possibility of modifying the invasive procedure influencing HRV. We

This work was carried out at Oita City Medical Association's Almeida Memorial Hospital, 1509-2 Miyazaki, Oita City, Oita, Japan.

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believe that clarifying the connection between autonomic regulation and pathophysiology in sepsis offers noninvasive and quick measurements of sepsis. Therefore, this study aimed to analyze and clarify them.

## METHODS

### Study design, setting, and participants

**T**HIS PROSPECTIVE, single-centered cohort trial was conducted between November 2019 and December 2020.

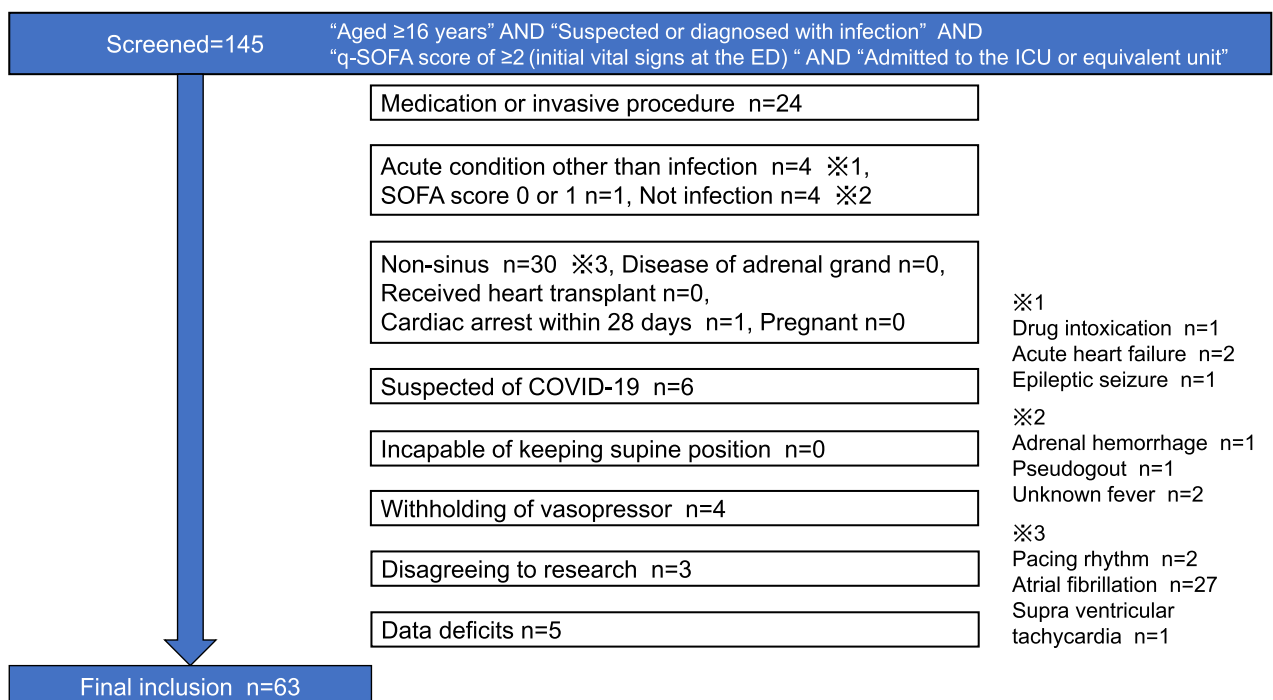
Patients aged  $\geq 16$  years who were suspected of or diagnosed with infection, satisfied the quick Sequential Organ Failure Assessment (q-SOFA) score of 2 or more<sup>8</sup> based on the initial vital signs observed at the emergency department (ED), and were admitted to the intensive care unit or equivalent unit were screened.

We screened using the q-SOFA score to prevent therapeutic modification of intervention to the autonomic parameters. For the same intent, patients who received any of the following medication or invasive procedures before measuring autonomic parameters were excluded: cardiovascular agents, sedative drugs, analgesics, neuromuscular blocking agents, intubation, invasive and noninvasive

positive pressure ventilation, drainage, debridement, and insertion of central venous or arterial catheters. To select patients with severe infection, those with acute conditions other than infection that might influence the q-SOFA, SOFA score of 0 or 1, or without infection at the final diagnosis (defined as without administration of therapeutic antibiotics use) were excluded. For technical limitations, patients whose cardiac rhythm was unsuitable for HRV measurement, including pacemaker rhythm, atrial fibrillation, and other nonsinus rhythms,<sup>9</sup> were excluded. Those who had a disease of the adrenal gland, received a heart transplantation,<sup>10</sup> suffered cardiac arrest within the last 28 days,<sup>11</sup> or were pregnant were also excluded. Furthermore, patients who were under suspicion of coronavirus disease 2019 (COVID-19) infection, unable to remain in a supine position during HRV measurement, had requested not to use vasopressors for resuscitative care, disagreed with inclusion in our research, or with data deficits were excluded.

### Variables and outcomes

We compared pathophysiological and clinical features of groups dichotomized by the cut-off values of autonomic parameters; high-frequency (HF) component of HRV as the



**Fig. 1.** Flowchart of the study population. COVID-19, coronavirus disease 2019; ED, emergency department; ICU: intensive care unit; q-SOFA: quick Sequential Organ Failure Assessment.

**Table 1.** Comparison of the characteristics of patients between subgroups with higher and lower autonomic parameters

Characteristics	All patients	Subgroups HF < 17.2 (ms <sup>2</sup> )	HF ≥ 17.2 (ms <sup>2</sup> )	P-value	Adrenaline < 0.11 (ng/mL)	Adrenaline ≥ 0.11 (ng/mL)	P-value
Pathophysiological and clinical parameter							
Patients, n	63	32	31		30	33	
Age, median (IQR)	82 (74–87)	82 (73–85)	85 (76–91)	0.134	83 (78–87)	82 (73–89)	0.625
Male, n (%)	26 (41)	11 (34.4)	15 (48.4)	0.311	15 (50)	11 (33.3)	0.208
Myocardial infarction, n (%)	2 (3)	2 (6.3)	0 (0)	0.492	1 (3.3)	1 (3)	>0.99
Diabetes mellitus, n (%)	19 (30)	13 (40.6)	6 (19.4)	0.0994	8 (26.7)	11 (33.3)	0.595
β-blocker use, n (%)	8 (13)	2 (6.3)	6 (19.4)	0.148	3 (10)	5 (15.2)	0.71
Body temperature (°C), mean ± SD	37.7 ± 1.3	38.0 ± 1.1	37.5 ± 1.4	0.115	37.9 ± 1	37.6 ± 1.5	0.468
Mean arterial pressure (mmHg), mean ± SD	79 ± 21	80 ± 24	78 ± 19	0.703	84 ± 22	74 ± 19	0.0572
Heart rate (beats/min), mean ± SD	102 ± 19	110 ± 15	94 ± 19	0.00032*	99 ± 15	106 ± 21	0.148
Respiratory rate (/min), median (IQR)	26 (23–30)	27 (24–32)	24 (22–28)	0.00948*	26 (23–28)	25 (23–30)	0.788
GCS total score, median (IQR)	13 (10–14)	12 (10–14)	13 (10–14)	0.772	13 (9–14)	13 (12–14)	0.739
P/F ratio, mean ± SD	273 ± 114	259 ± 104	287 ± 125	0.339	292 ± 101	255 ± 124	0.207
Creatinine (mg/dL), median (IQR)	1.26 (0.79–2.92)	0.99 (0.73–2.41)	1.28 (0.93–3.07)	0.163	1.02 (0.75–1.98)	1.86 (0.88–3.15)	0.077
Total bilirubin (mg/dL), median (IQR)	0.6 (0.5–1)	0.7 (0.5–1)	0.6 (0.5–1)	0.379	0.6 (0.5–0.8)	0.7 (0.5–1.1)	0.116
Platelet (10 <sup>4</sup> /μL), mean ± SD	18.9 ± 9.7	19.5 ± 9.7	18.3 ± 9.8	0.609	19.4 ± 9.4	18.5 ± 10.1	0.714
CRP (mg/dL), median (IQR)	9.75 (3.26–17.7)	11.34 (5–18.01)	9.15 (3.26–16.73)	0.508	8.56 (2.86–17.18)	10.26 (5.71–17.96)	0.416
Bacteremia, n (%)	18 (29)	14 (43.8)	4 (12.9)	0.0111*	7 (23.3)	11 (33.3)	0.416
Lactate (mmol/L), median (IQR)	2.7 (1.4–4.8)	3.0 (1.9–5.4)	2.6 (1.4–3.9)	0.274	1.8 (1.1–3)	4.1 (2.2–7)	0.00143*
SIRS score ≥ 2, n (%)	60 (95)	32 (100)	28 (90.3)	0.113	29 (96.7)	31 (93.9)	>0.99
SOFA score, median (IQR)	4 (5–7)	5 (4–6)	5 (4–7)	0.829	4 (3–6)	6 (4–8)	0.0145*
VD-48, n (%)	26 (41)	12 (37.5)	14 (45.2)	0.613	6 (20)	20 (60.6)	0.00187*
MV, n (%)	12 (19)	6 (18.8)	6 (19.4)	>0.99	4 (13.3)	8 (24.2)	0.344
De novo RRT, n (%)	8 (13)	2 (6.3)	6 (19.4)	0.148	0 (0)	8 (24.2)	0.0051*
IHM, n (%)	12 (19)	5 (15.6)	7 (22.6)	0.536	2 (6.7)	10 (30.3)	0.0241*
Baseline autonomic parameter							
HF (ms <sup>2</sup> ), median (IQR)	14 (4.1–66.3)						
LF/(LF + HF), mean ± SD	0.554 ± 0.2						
Adrenaline (ng/mL), median (IQR)	0.11 (0.05–0.37)						
Details of pathogens							
<i>Escherichia coli</i>	8						
<i>Klebsiella pneumoniae</i>	4						
Coagulase-negative staphylococcus	2						
Group G <i>Streptococcus</i>	1						
<i>Peptostreptococcus</i> sp.	1						
	1						

**Table 1.** (Continued)

Characteristics	All patients	Subgroups		P-value	Adrenaline < 0.11 (ng/mL)	Adrenaline ≥ 0.11 (ng/mL)	P-value
		HF < 17.2 (ms <sup>2</sup> )	HF ≥ 17.2 (ms <sup>2</sup> )				
<i>Pseudomonas fluorescens</i>							
<i>Acinetobacter lwoffii</i>						1	

Subgroups were dichotomized by cut-off values calculated in accordance with area under the curve.

CRP, C-reactive protein; GCS, Glasgow Coma Scale; HF, high frequency; IHM, in-hospital mortality; IQR, interquartile range; LF, low frequency; MV, mechanical ventilation; P/F, partial pressure of arterial oxygen tension-to-inspiratory oxygen fraction ratio; RRT, renal replacement therapy; SD, standard deviation; SIRS, systemic inflammatory response syndrome; SOFA, Sequential Organ Failure Assessment; VD-48, vasopressor dependence during 48 h after arrival.

\* $P < 0.05$ .

representative parameter of PNS, LF-to-HF + LF (low-frequency) component ratio as SNS,<sup>9</sup> and serum adrenaline concentration as SAS.

The primary outcome was vasopressor dependence during 48 h after arrival (VD-48). The secondary outcomes were the presence of bacteremia,<sup>12,13</sup> mechanical ventilation (MV) use (invasive or noninvasive positive pressure ventilation), application of *de novo* renal replacement therapy (RRT; continuous or intermittent), and in-hospital mortality. Bacteremia was diagnosed retrospectively by a principal investigator. Contamination was considered when (i) *Corynebacterium* spp., *Bacillus* spp., or *Propionibacterium acnes* were detected, or (ii) coagulase-negative staphylococci, viridans group streptococci, or *Clostridium perfringens* were detected only in a single set of bottles.<sup>14</sup>

### HRV and serum adrenaline measurements at the ED

HRV and serum adrenaline were measured immediately to prevent modification of intervention to autonomic parameters.

The technique used to measure HRV followed the Taskforce of the European Society of Cardiology and North American Society of Pacing and Electrophysiology guidelines.<sup>9</sup> The terminal device for measuring HRV was the Holter ECG recorder (NIHON KOHDEN RAC-5203, NIHON KOHDEN, Tokyo, Japan), and the analytical software was SUWA TRUST DSC-5500 MemCalc/Chiram 3 (SUWA TRUST, Tokyo, Japan). The terminal device was attached to each patient by a trained medical laboratory technologist (MLT). The first 5-min data were adopted for HRV calculation, with spine position assessment and visual inspection by the MLT. Extrasystoles or noises were manually deleted from the original data series by the MLT.

Blood specimens for measuring adrenaline were centrifuged as soon as possible, and serum samples were frozen at  $-30^{\circ}\text{C}$ .

Clinicians were blinded to the results of HRV and serum adrenaline.

### Other data sources

We collected the following medical information: age, sex, medical history of myocardial infarction,<sup>15</sup> diabetes mellitus,<sup>16</sup> the use of  $\beta$ -blocker,<sup>17</sup> initial vital signs, and laboratory data.

### Statistical analysis

Serum adrenaline less than the lower limit of quantification was substituted for lower limit of quantification values.

Statistical significance was considered when the two-sided  $P$ -value was  $<0.05$ . The normality of each continuous variable was assessed using the Shapiro–Wilk test. Data of normal distribution variables were expressed as mean and standard deviation and compared between groups using the Student's  $t$ -test. Data of non-normal distribution were expressed as a median and interquartile range and compared using the Mann–Whitney  $U$  test. Categorical data were compared using Fisher's exact probability test.

The optimal cut-off value of the autonomic parameter corresponding to each outcome was defined using the Youden index method in the receiver operating characteristic (ROC) analysis. The subgroup was tested using logistic regression analysis and expressed as odds ratio (OR) with a 95% confidence interval (CI).

The correlation between each autonomic parameter was measured using the Pearson correlation coefficient.

Statistical analysis was performed using R version 3.5.2 (R Foundation for Statistical Computing, Vienna, Austria).

## RESULTS

A TOTAL OF 145 patients were screened, of whom 63 were finally included in the analysis, as illustrated in Figure 1. All the patients had SOFA score of 2 or more on admission.

Characteristics of study patients are presented in Table 1.

In the ROC plane, VD-48, *de novo* RRT, and in-hospital mortality had the strongest association with higher adrenaline (cut-off 0.11, 0.11, and 0.12 ng/mL, respectively), and bacteremia had the strongest association with lower HF (17.2 ms<sup>2</sup>). MV use was associated with LF-to-HF + LF ratio, with a low area under ROC (Table 2). Comparison between subgroups dichotomized by adrenaline of 0.11 ng/mL and HF of 17.2 ms<sup>2</sup> is presented in Table 1.

In the multivariate logistic regression model adjusted for age and sex, VD-48 remained highly associated with adrenaline ( $\geq 0.11$  ng/mL; OR 9.71, 95% CI 2.55–37;  $P = 0.000874$ ), and bacteremia with lower HF ( $< 17.2$  ms<sup>2</sup>; OR 4.86, CI 1.36–17.4;  $P = 0.0152$ ; Table 3).

We evaluated the correlation between (i) (LF to HF + LF) – HF (ii) (LF to HF + LF) – adrenaline, and (iii) HF – adrenaline. There were no significant correlations between them (Fig. 2).

## DISCUSSION

THIS STUDY REVEALED the integral association of the autonomic regulator with pathophysiological or clinical change in one cohort with suspected sepsis. Our results show that circulatory insufficiency, organ dysfunction, and mortality were associated with SAS, and bacteremia was associated with decreased PNS, based on the result of serum adrenaline and the 5-min measurement of HRV at the ED.

This is the only study so far that revealed the relationship between HRV and the presence of bacteremia in adults. *In vitro*, the causal relation between PNS and inflammatory response (inflammatory reflex) was suggested as the vagus nerve stimulation attenuating the systemic inflammatory response.<sup>18</sup> A previous study indicated the association between higher interleukin-6 and bacteremia.<sup>19</sup> HF was found to be negatively correlated with interleukin-6 in patients with sepsis with hypercytokinemia by Tateishi *et al.*<sup>20</sup> These studies conform to the findings of our study.

In this study, we could not show the relationship between HRV parameters and the mortality or severity of sepsis compared with previous studies.<sup>5–7</sup> The limitations to the

**Table 2.** AUC and the cut-off value of autonomic parameters corresponding to each outcome

Outcome	HF (ms <sup>2</sup> )			LF/(LF + HF)			Adrenaline (ng/mL)					
	AUC (95% CI)	Sensitivity	Specificity	Cut-off value	AUC (95% CI)	Sensitivity	Specificity	Cut-off value	AUC (95% CI)	Sensitivity	Specificity	Cut-off value
VD-48	0.52 (0.369–0.67)	0.192	0.919	160.5	0.516 (0.365–0.666)	0.192	0.946	0.286	0.751 (0.629–0.873) <sup>†</sup>	0.769	0.649	0.11
Bacteremia	0.735 (0.601–0.87) <sup>†</sup>	0.833	0.6	17.2	0.526 (0.354–0.698)	0.444	0.756	0.667	0.532 (0.387–0.678)	0.833	0.378	0.07
MV	0.474 (0.298–0.649)	1	0.118	176.1	0.68 (0.49–0.87) <sup>†</sup>	0.583	0.765	0.421	0.651 (0.473–0.829)	0.333	0.922	1
<i>De novo</i> RRT	0.736 (0.553–0.92)	1	0.436	8.6	0.632 (0.39–0.873)	0.375	0.927	0.286	0.806 (0.667–0.944) <sup>†</sup>	1	0.545	0.11
IHM	0.59 (0.375–0.805)	0.922	0.417	109.6	0.673 (0.502–0.844)	0.824	0.5	0.414	0.746 (0.577–0.915) <sup>†</sup>	0.627	0.833	0.12

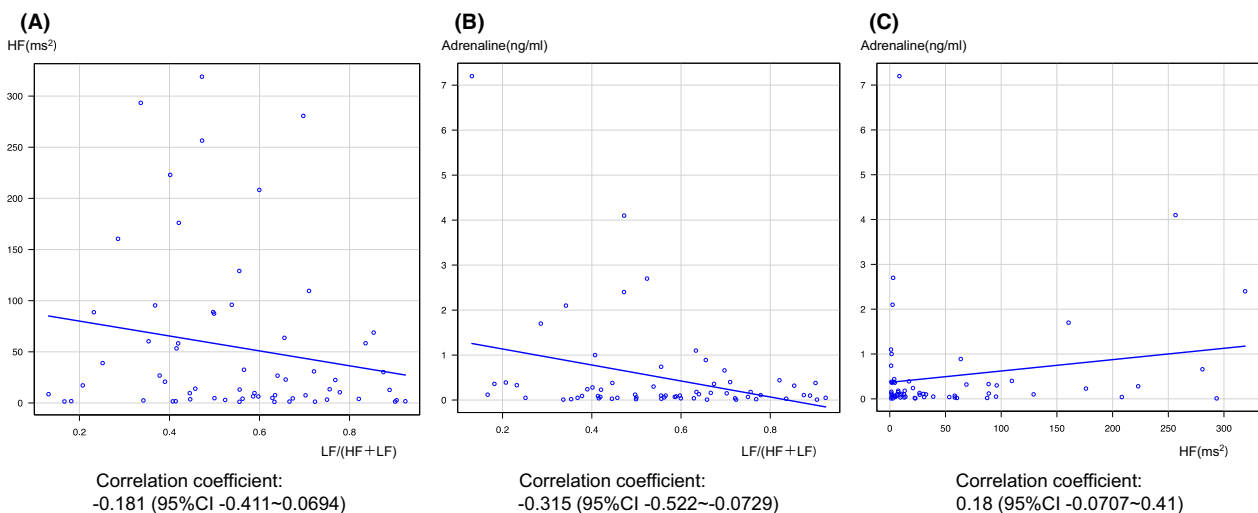
AUC, area under the curve; CI, confidence interval; HF, high frequency; IHM, in-hospital mortality; LF, low frequency; MV, mechanical ventilation; RRT, renal replacement therapy; VD-48, vasopressor dependence during 48 h after arrival.

<sup>†</sup> Largest AUC value of each outcome.

**Table 3.** Multivariate logistic regression model for VD-48 and bacteremia

For VD-48	Univariable		Multivariable	
	OR (95% CI)	P-value	OR (95% CI)	P-value
Age	0.954 (0.911–0.998)	0.0417*	0.941 (0.894–0.989)	0.0174*
Sex (Male)	1.41 (0.509–3.9)	0.51	2.89 (0.787–10.6)	0.11
Adrenaline $\geq 0.11$ (ng/mL)	6.15 (1.98–19.1)	0.0017*	9.71 (2.55–37)	0.000874*
For bacteremia	Univariable		Multivariable	
	OR	P-value	OR	P-value
Age	0.976 (0.934–1.02)	0.294	0.984 (0.937–1.03)	0.512
Sex (Male)	0.625 (0.199–1.96)	0.42	0.779 (0.229–2.65)	0.689
HF < 17.2 (ms <sup>2</sup> )	5.25 (1.49–18.5)	0.00996*	4.86 (1.36–17.4)	0.0152*

CI, confidence interval; HF, high frequency; OR, odds ratio; VD-48: vasopressor dependence during 48 h after arrival.  
\* $P < 0.05$ .

**Fig. 2.** Correlation between (A) (LF to HF + LF) – HF, (B) (LF to HF + LF) – adrenaline, and (C) HF – adrenaline. CI, confidence interval; HF, high frequency; LF, low frequency.

HRV interpretation as a representative of autonomic regulation in sepsis are considered. First, invasive procedures influence HRV. Second, the SNS is regulated by controlling different groups of premotor neurons in parallel,<sup>21</sup> and HRV, which is reflective of cardiac sympathetic nerve activity, is not always reflective of other organs. Third, HRV is a dynamic and transient parameter of the SNS and PNS activity,<sup>22</sup> and changes in short-term HRV measurement are too simple to interpret as a complicated mechanism of multiple organ dysfunction.

In our study, the respiratory frequency or heart rate of patients could not be controlled, which might have influenced the HF band.<sup>23</sup> In addition, the influence of medical

procedures that are slightly invasive comparatively on HRV has not been gauged, such as blood sampling, peripheral intravenous infusion, and conversation. While considering these unavoidable factors in an actual clinical situation, HRV will help provide new monitoring guidelines for early stage sepsis in which PNS has an important modulative effect on inflammation, bacteremia, or viremia such as COVID-19.<sup>24</sup>

In addition, considering that the connection between SNS, PNS, and SAS was not established in this study, the autonomic regulator may involve a multilayered and differentiated modulating process for each pathophysiological element of sepsis, as mentioned in a previous study.<sup>4</sup> The



SAS may be an important modulator of late-stage sepsis and one of the important therapeutic targets, such as sepsis-induced cardiac dysfunction by excessive stimulation of sympathetic effect.<sup>25</sup>

This study has limitations. First, cases excluded due to invasive procedures before HRV or serum adrenaline measurements are thought to be more severe than those included. Previous research indicated that sepsis with atrial fibrillation, in which HRV cannot be measured, is more severe than sepsis without atrial fibrillation.<sup>26</sup> Second, q-SOFA as an inclusion criterion consisted of initial vital signs taken at the ED, which is different from previous major studies that included the worst vital sign at the ED.<sup>27</sup> Third, diagnosing or suspecting infection depended on the proficiency of the individual clinician, and all study participants had not been strictly diagnosed with sepsis. In our study, the “elevation” of SOFA score of 2 or more<sup>8</sup> was inestimable in 62% (39 cases) of included patients due to insufficient data about the second point of SOFA. However, all the patients had both q-SOFA and SOFA scores of 2 or more, and systemic inflammatory response syndrome scores of 2 or more were 95% (60 cases) in our study. For considering past research which revealed SOFA score of 2 or more was associated with worse outcome of sepsis,<sup>27,28</sup> included patients in this study had a certain severity of sepsis. Fourth, the geriatric population was more predominant than previously reported.<sup>27</sup> Finally, this was an exploratory, single-institution study with a small sample size.

## CONCLUSIONS

CIRCULATORY INSUFFICIENCY, organ dysfunction, and mortality were associated with SAS activity, and bacteremia was associated with decreased PNS activity. The autonomic regulator modulating pathophysiology of sepsis may vary depending on the multilayered and differentiated modulating process. Further studies are needed to clarify them.

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## DISCLOSURE

APPROVAL OF THE research protocol with approval No. and committee Name: This study protocol

complies with the Declaration of Helsinki and was approved by the institutional review boards at Oita City Medical Association's Almeida Memorial Hospital (approval no: 158).

Informed Consent: Written informed consent was obtained from all patients or from their legally authorized representatives.

Registry and the Registration No. of the study/Trial: This trial was registered at University Hospital Medical Information Network (UMIN000038458) on 4/11/2019.

Animal Studies: N/A

Conflict of Interest: None declared.

## DATA AVAILABILITY STATEMENT

THE DATA SETS used and analyzed during this current study are available from the corresponding author on reasonable request.

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## SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

**Fig. S1** Scheme of heart rate variability (HRV) analysis. From the analysis of time series of R–R intervals on electrocardiogram (ECG) by the fast Fourier transform (FFT) or maximum entropy method (MEM), high-frequency (HF) and low-frequency (LF) components of HRV are obtained. The data shown are derived from the measurement by the corresponding author.