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A novel biomarker of serum Histidine-Rich Glycoprotein (HRG) for diagnosing and predicting prognosis of ventilator-associated pneumonia (VAP): a pilot study

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Abstract. – OBJECTIVE: Histidine-Rich Glycoprotein (HRG) has been reported to be associated with idiopathic pulmonary fibrosis, cancer, and sepsis as a novel biomarker. However, there is limited evidence regarding its value in diagnosing or prognosis evaluating of ventilator-associated pneumonia (VAP).

PATIENTS AND METHODS: A total of 186 patients intubated in ICU and 65 healthy volunteers were enrolled in this study. Patients were divided into VAP group (n = 116), non-VAP group (n = 70) and control group (n = 65). The HRG, C reactive protein (CRP) and procalcitonin (PCT) levels were measured 72 hours after intubation, while blood sample was acquired from healthy controls for the test.

RESULTS: HRG of VAP group was significantly lower than non-VAP group and control group ($p < 0.001$), while CRP and PCT were significantly higher ($p < 0.001$). The ROC analysis showed that the AUC of HRG was 0.777 95% CI (0.708-0.847) with a cut-off value of 38.55 μg/mL, which was lower than CRP [AUC = 0.912, 95% CI (0.847-0.950)] and PCT [AUC = 0.818, 95% CI (0.759-0.876)]. No linear correlation was found between HRG and CRP, as well as PCT ($p > 0.05$). However, the survival analysis showed that patients with higher level of HRG had a significantly higher survival rate ($p < 0.001$). The multivariate Cox regression analysis also demonstrated that the higher level of HRG was associated with better survival [HR 0.290, 95% CI (0.131-0.641), $p = 0.002$].

CONCLUSIONS: Serum HRG decreases when the patient develops VAP, which might be used as a biomarker for the diagnosis of VAP, with relatively less accuracy than PCT and CRP. However, HRG is valuable in predicting the clinical outcomes of mechanical ventilation patients.

Key Words:
Histidine-Rich Glycoprotein (HRG), Ventilator-associated pneumonia, Diagnosis, Prognosis.

Introduction

Ventilator-associated pneumonia (VAP) is one of the most common types of infections acquired in hospital, which happens in intubated patients[1]. Despite of multiple prevention measures taken in ICU[2], VAP still dominates as one of the major threatening complications[3]. Bacterial spectrum varies with the application of new generation antibiotics, when multidrug-resistant organisms occupies more than half of all pathogens of population, and greatly increase the mortality[4]. Early diagnosis, accurate classification and proper estimates prognosis may help preventing and promoting the clinical outcomes of VAP[5]. Numerous biomarkers including[25,26] C reactive protein (CRP), procalcitonin (PCT), and endotoxin have been found or used as tools to diagnose or judge the severity of VAP[6]. However, the present biomarkers show disadvantages such as low specificity, limitation of G+ bacteria and so on[7], which necessitate development and validation of new biomarkers for VAP. Physiologically, Histidine-rich glycoprotein (HRG) is a multi-domain (structured) protein produced by liver and involved in modulation of immune/autoimmune, vascular, fibrinolysis and coagulation systems[8,9]. It is also associated with some kinds of pathological processes an diseases, including inflammation[10], cancer[11], and sepsis[12]. However, there is rarely application research on its value as a prac-

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tical biomarker, especially for bacterial infection. In this prospective observational cohort studies, we examined the diagnostic and prognostic value of HRG for VAP.

Patients and Methods

Patient Enrollment

This prospective observational cohort study was approved by the Ethical Committee of our Hospital, and informed consent was obtained from each subject. Patients admitted to the Intensive Care Unit (ICU) of Emergency Room of Hospital were enrolled in this study if they met the following criteria. Inclusion criteria: the patient must be adult (age over 18 years old); must be intubated with mechanical ventilation; the patient or family must agree to participate in the study and sign the informed consent. Exclusion criteria: patients in extremely critical condition with expected mortality were excluded; patients with tumor, infection in other system, or history of auto-immune disease were excluded; patients with pregnancy, hepatic dysfunction, heart failure or renal dysfunction were excluded. Healthy volunteers were recruited and compensated in this study.

Sample Collection

Since there is no universally accepted gold standard diagnostic criterion for VAP, a comprehensive diagnosis tool using clinical, physiological, microbiological and radiographic evidence (Clinical Pulmonary Infection Score, CPIS) was adopted in this study. When the CPIS score exceeded 6 points, the patient was diagnosed with VAP[13]. After 72 hours admission into ICU, the patients were divided into VAP group (CPIS > 6 points) and non-VAP group (CPIS $\leq$ 6 points), when blood sample was collected. Blood sample was also collected from the healthy volunteers (Control group). The blood sample was centrifuged (3500 rpm, 10 min) immediately and the supernatant was collected and stored at -80°C. Serum HRG level was measured using ELISA Kit (Biomatik, EKU04805), while CRP and procalcitonin (PCT) were measured using automated enhanced chemiluminescence immune analyzer (Modular Analytics E-170; Roche Diagnostics, Mannheim, Germany) in the laboratory of the hospital.

Data Collection

Demographical characteristics were collected from all participants including volunteers. Clinical data including the patient type (medical or surgical), disease severity (SOFA score, APACHE II score, and SAPS II score at admission), special treatment (Vasopressors, Blood purification, and Corticosteroids), ventilation time, and ICU stay, were collected from the electronic medical record system of our hospital. In-hospital death (30 days) was also recorded.

Statistical Analysis

In this study, IBM SPSS Statistics, version 19.0 (IBM, Armonk, NY, USA) was used for statistical analysis. Normality distribution test of the variables was conducted first to check the variables distribution condition. Continuous variables meeting the normal distribution were presented as mean ± standard deviations and categorical variables were presented as proportions, while continuous variables unfitting the normal distribution were described as median and interquartile range (IQR). Demographical characteristics and clinical data of three groups were compared. Comparison of continuous variable of different groups was conducted with *t*-test of independent samples. x^2-test was performed in different evaluations of categorical variables. Mann-Whitney test and Kruskal-Wallis test were adopted for the comparison of different groups in non-normal variables of independent samples. Receiver operating characteristic (ROC) curve analysis was used to evaluate the value of serum HRG, CRP, PCT level in diagnosing VAP. Cut-off value was determined when the Youden Index was highest. A linear regression between HRG and other inflammatory factors was conducted. Kaplan-Meier survivals curves and log rank tests were used to compare the survival status of patients with higher or lower level of HRG (Figure 1). Cox hazard ratio model was used to analyze the risk factors for the survival of ventilated patients. Variates were chosen into the multivariate model only when the *p*-value was lower than 0.10 and the "enter" mode of the parameters was set. *p*-value < 0.05 was considered as statistical significant.

Results

Demographical Characteristics and Clinical Data

Table I showed the demographical characteristics and clinical data of three groups. There was no significant difference between three groups in

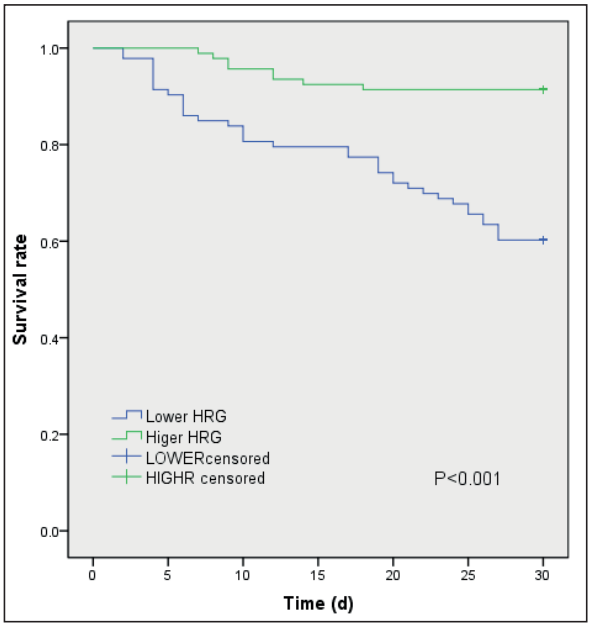

Figure 1. Serum level of biomarkers in three groups. ***(a)*** Serum CRP level of CON group, non-VAP group and VAP group; ***(b)*** Serum PCT level of CON group, non-VAP group and VAP group; c) Serum HRG level of CON group, non-VAP group and VAP group; *$p < 0.001$.

demographical characteristics (all $p > 0.05$). Patients' type differed between non-VAP and VAP group, without statistical difference ($p = 0.067$). However patients of VAP group had much more severe conditions than the non-VAP group, with significantly higher all three scores (all $p < 0.001$). Therefore, more special treatment was given for the VAP group including vasopressors ($p = 0.850$), blood purification ($p = 0.075$), and corticosteroids ($p = 0.023$), and VAP group had longer ventilation time and ICU stay ($p < 0.001$). However, there was no significantly difference between two groups in in-hospital death rate ($p = 0.216$). Figure 2 showed the biomarker level of three groups. There was significant difference between three groups as well as each two groups in HRG, CRP, PCT level (all $p < 0.001$). As shown in Figure 3 and Table II, there was no significant correlation between HRG, CRP, and PCT level (all $p > 0.05$).

Diagnosis for VAP

As shown in Figure 3, three biomarkers of HRG, CRP, PCT were compared in their accuracy of diagnosing VAP. With a cut-off value of 38.55 μg/ml, the HRG showed sensitivity of 0.996 and specificity of 0.500, and the AUC of HRG was 0.777 95% CI (0.708-0.847). As for the PCT, the sensitivity was 0.578 and the specificity was 1.000 with a cut-off value of 1.946 ng/ml. The AUC of PCT was 0.818, 95% CI (0.759-0.876). The CRP showed best accuracy of diagnosing VAP, its sensitivity was 0.759 and the specificity was 0.957 with a cut-off value of 108.35 mg/L; its AUC was 0.912, 95% CI (0.847-0.950).

Table I. Demographical characteristics and clinical data of three groups.

Variables	Control group (n 65)	Non-VAP group (n = 70)	VAP group (n = 116)	*p*-value
Demographics				
Age (y, Mean ± SD)	49.0 ± 19.0	50.6 ± 15.3	54.3 ± 17.1	0.101
Gender (% male)	38 (58.5%)	49 (70.0%)	85 (73.3%)	0.114
BMI (kg/m^2)	22.8 ± 4.1	23.1 ± 4.3	23.5 ± 4.0	0.581
Smoking (%)	13 (20.0%)	19 (27.1%)	36 (31.0%)	0.277
Patients type				0.067
Surgical patients (%)	NA	46 (65.7%)	59 (34.3%)	
Medical patients (%)	NA	24 (50.9%)	57 (49.1%)	
Severity of disease				
SOFA score (IQR)	NA	8 (5-12)	12.5 (9-15)	< 0.001
APACHE II score (IQR)	NA	14 (12-18)	20 (16-25)	< 0.001
SAPS II score (IQR)	NA	39.5 (30-49)	47 (37-60)	< 0.001
Special treatment				
Vasopressors (%)	NA	13 (18.6%)	24 (20.7%)	0.850
Blood purification (%)	NA	5 (7.1%)	19 (16.4%)	0.075
Corticosteroids (%)	NA	11 (15.7%)	36 (31.0%)	0.023
Ventilation time (d, IQR)	NA	3 (2-4)	9 (5-11)	< 0.001
ICU stay (d, IQR)	NA	9 (5-11)	16 (10-23)	< 0.001
In-hospital death (%)	NA	13 (18.6%)	32 (27.6%)	0.216

BMI: body mass index; NA: not applicable.

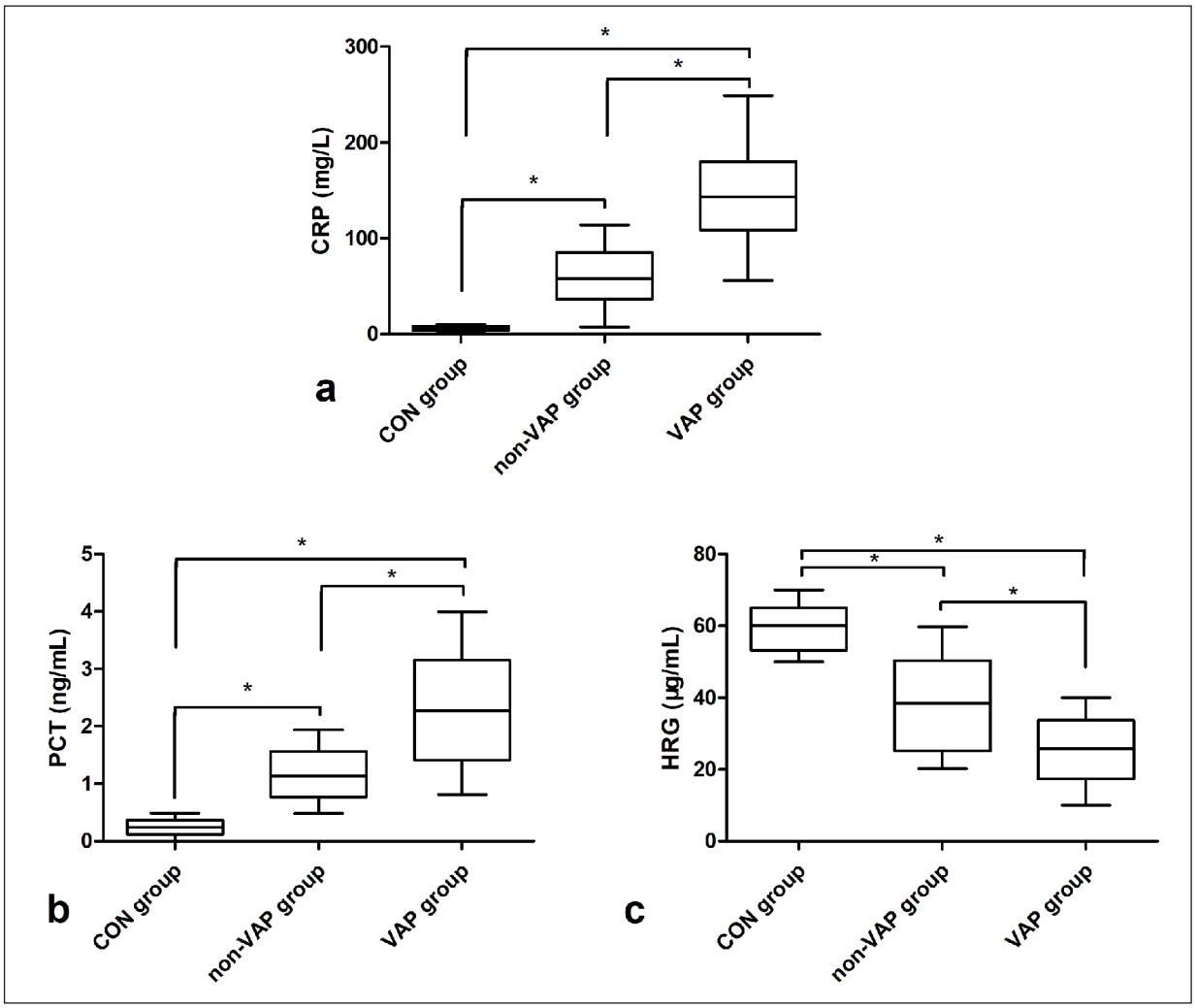

Figure 2. Linear regression of serum HRG and other inflammatory factors. ***(a)*** linear regression between HRG and CRP in control group; ***(b)*** linear regression between HRG and PCT in control group; c) linear regression between HRG and CRP in non-VAP group; ***(d)*** linear regression between HRG and PCT in non-VAP group; ***(e)*** linear regression between HRG and CRP in VAP group; ***(f)*** linear regression between HRG and PCT in VAP group.

Predicting Prognosis for VAP

When all patients were divided into lower HRG group (HRG < 29.5 μg/ml) and higher HRG group (HRG ≥ 29.5 μg/ml), the survival rates were compared between two groups using Kaplan-Meier curve and log rank test (Figure 4). The higher HRG group survived longer than lower HRG group ($p < 0.001$). Moreover, Table III and Table IV demonstrated the Cox regression analysis results with univariate or multivariate respectively, validating the strong association between HRG level and the survival of the patients ($p < 0.001$). It also showed that the age and SOFA score were the independent risk factors for the mortality of the patients ($p < 0.001$).

Discussion

Highly occurring in ICU patients, VAP also significantly burdens patients with increased mortality and cost[14], making it a clinically research hotpot. Using a prospective observational cohort, this study mainly found the following results: 1. Serum HRG levels were significantly lower in the VAP patients than those patients without VAP, and both were lower than the healthy controls; 2. HRG can be used as a diagnostic biomarker, while the accuracy was not as good as CRP or PCT; 3. Patients with higher HRG level showed a better survival condition than those with lower HRG level. Same as other

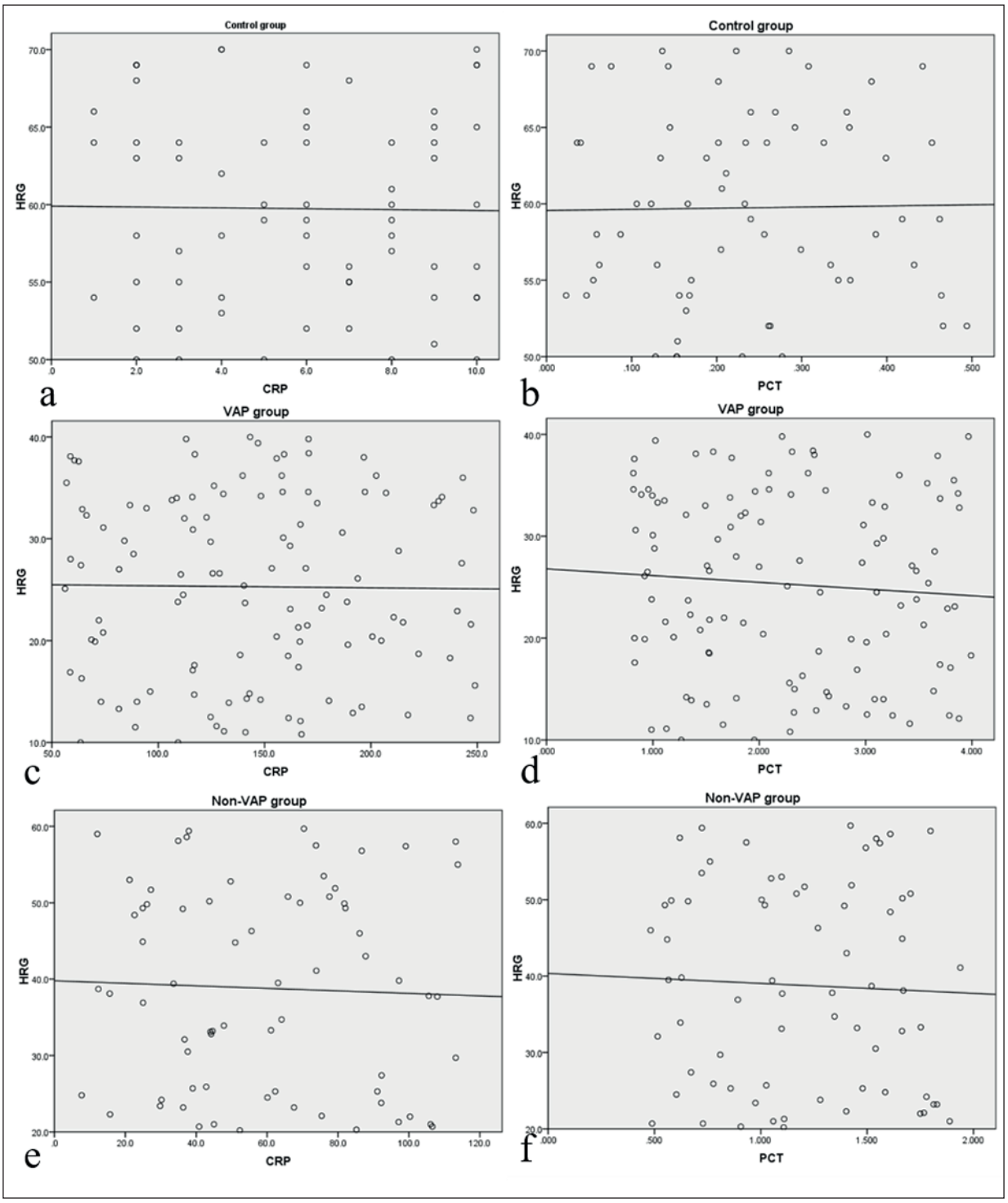

Figure 3. ROC curve of HRG, CRP and PCT in diagnosing VAP.

nosocomial infection, the most effective way of handling VAP lies in prevention. A bundle of preventing measures has been developed, including prevention of aerodigestive tract colonization (avoidance of unnecessary antibiotics and stress ulcer prophylaxis, use of sucralfate for stress ulcer prophylaxis, chlorhexidine oral rinse, selective digestive decontamination, short-course parenteral prophylactic antibiotics in high-risk patients) and the prevention of aspi-

Table II. Linear correlation analysis between HRG and other inflammatory factors.

Independent variable	Dependent variable	HRG		
		Control group (n = 65)	Non-VAP group (n = 70)	VAP group (n = 116)
PCT	Constant	59.566	40.342	26.791
	b	0.743	-1.303	-0.659
	R^2	< 0.001	0.002	0.005
	P	0.904	0.725	0.438
CRP	Constant	59.905	39.777	25.579
	b	-0.028	-0.016	-0.002
	R^2	< 0.001	0.001	< 0.001
	P	0.915	0.760	0.899

HRG: Histidine-Rich Glycoprotein; PCT: procalcitonin; CRP: C reactive protein.

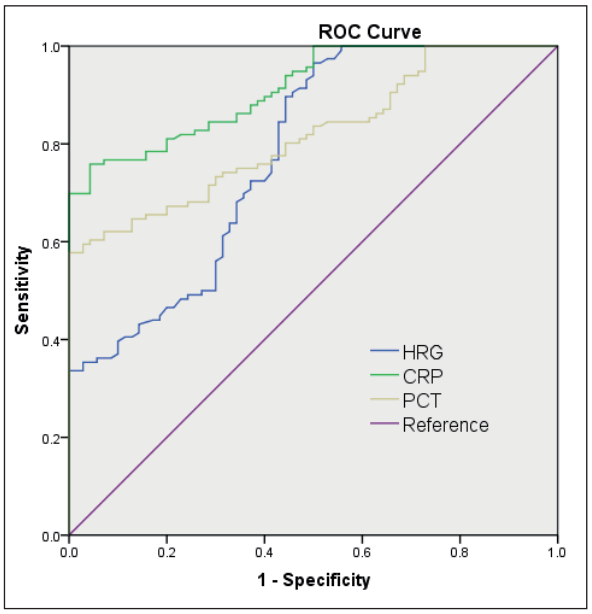

Figure 4. Survival curve of patients with lower level of HRG and higher level of HRG.

ration of contaminated secretions (preferred oral intubation, appropriate intensive care unit staffing, avoidance of tracheal intubation with the use of mask ventilation, application of weaning protocols and optimal use of sedation to shorten the duration of mechanical ventilation, semirecumbent positioning, minimization of gastric distension, subglottic suctioning, avoidance of ventilator circuit changes/manipulation, routine drainage of ventilator circuit condensate)[15]. Despite of the effect of prevention bundles, VAP remains one of the most frequent hospital-acquired infections occurring in intubated patients, making early diagnosis and accurate evaluation crucial for the rapid and proper antibiotic therapy[16]. However, diagnosis of VAP can be relatively difficult without a gold standard, and the major diagnostic measures can be divided into invasive and non-invasive techniques[14]. Biomarkers are one of the most common non-in-

Table III. Univariate Cox regression analysis of risk factors and patients' survival.

Variates	B	SE	Wald	HR	95% CI	p
AGE	0.032	0.009	11.251	1.032	1.013-1.052	0.001
Gender (male to female)	-0.191	0.322	0.351	0.826	0.440-1.553	0.554
BMI	0.061	0.038	2.663	1.063	0.988-1.145	0.103
Smoking	0.635	0.302	4.417	1.887	1.044-3.410	0.036
Type (Medical to Surgical)	0.359	0.298	1.448	1.432	0.798-2.570	0.229
SOFA	0.234	0.042	30.973	1.264	1.164-1.373	< 0.001
APACHE	0.057	0.026	4.940	1.059	1.007-1.114	0.026
SAPS	0.039	0.013	9.160	1.040	1.014-1.067	0.002
Vasopressors	0.599	0.329	3.313	1.821	0.955-3.471	0.069
Blood Purification	-0.504	0.524	0.926	0.604	0.216-1.686	0.336
Corticosteroids	-0.061	0.347	0.031	0.941	0.477-1.857	0.861
HRG level (Higher to lower)	-1.699	0.390	18.949	0.183	0.085-0.393	< 0.001

BMI: body mass index; NA: not applicable.

Table IV. Univariate Cox regression analysis of risk factors and patients' survival.

Variates	B	SE	Wald	HR	95% CI	p
AGE	0.032	0.010	10.043	1.032	1.012-1.053	0.002
Smoking	0.323	0.314	1.057	1.381	0.746-2.554	0.304
SOFA	0.203	0.044	21.086	1.225	1.123-1.336	< 0.001
APACHE	-0.016	0.029	0.315	0.984	0.931-1.041	0.574
SAPS	0.016	0.011	1.897	1.016	0.993-1.039	0.168
Vasopressors	0.299	0.334	0.803	1.349	0.701-2.594	0.370
HRG level (higher to lower)	-1.239	0.406	9.334	0.290	0.131-0.641	0.002

vasive techniques for diagnosing VAP. CRP and PCT are the most widely studied biomarkers for diagnosis and evaluation of nosocomial infection including VAP[17,18]. Povoa et al[19] found that for patients under mechanical ventilation, daily CRP monitoring was useful in VAP prediction, while PCT showed a poor predictive performance[19]. Similarly to Povoa et al[19] results, Habib et al[20] demonstrated positive results of CRP and an insignificant result of PCT in diagnosing CRP[20]. However, Jiao et al[21] analyzed the value of PCT in diagnosing VAP for patients undergoing cardiac surgery, and the results showed that serum PCT might be used as diagnostic marker for VAP with a sensitivity of 91% and a specificity of 71%. Those discrepancies can be attributed to the different test time, population and the criteria for VAP. Our study also examined the diagnostic value of CRP and PCT as the comparative standard for HRG, and the results showed the best performance of CRP, a medium performance of PCT and last performance of HRG. However, a previous study focusing on HRG and sepsis found that HRG worked well as biomarker for sepsis among Systemic Inflammatory Response Syndrome (SIRS), with an area under the curve (AUC) of 0.97[22]. The difference of HRG in sepsis and VAP could be attributed to the difference between systematic infection and local infection[23]. Another focused research topic is the evaluation or prognosis predicting using biomarkers. A study explored the association between CRP, PCT levels and survival condition of VAP patients, and the results were negative[24]. This work compared the survival condition between patients with lower HRG level and higher HRG level, and there was a significant difference, which implied the value of HRG for predicting the severity and survival of VAP patients. Despite of the prospective design and convincing results, several limitations must be noted. First of all, this was a single center study with relatively small sample size, which required further validation of larger scale studies. Second, the blood sample was acquired at only one point, so the trend of biomarkers was not observed. Last, CPIS score standard was adopted in this study; it was not gold standard and had limited sensitivity and specificity.

Conclusions

We demonstrated a significantly difference of HRG between VAP patients, non-VAP patients and healthy population, which might be used as a biomarker for the diagnosis of the VAP, with relatively less accuracy than PCT and CRP. However, HRG is valuable in predicting the clinical outcomes of mechanical ventilation patients. Future studies should expand the sample size, participants of different centers, and add the test time of HRG.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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