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Serum histidine-rich glycoprotein versus sTREM-1 in early detection of ventilator-associated pneumonia in intensive care unit adult patients: A pilot study

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ABSTRACT

Background: Ventilator-associated pneumonia (VAP) is a serious infection that affects patients on ventilators in intensive care units (ICUs). Early diagnosis and treatment are crucial to improve outcomes. Researchers are looking for new biomarkers to help diagnose and manage VAP, including HRG and Triggering Receptor Expressed on Myeloid cells-1 (TREM-1), which have shown promise in predicting disease severity and outcomes. This study aimed to investigate and compare the diagnostic and prognostic utility of sTREM-1 and HRG in adult ICU patients with ventilator-associated pneumonia, filling a current knowledge gap in the field. **Method**: A total of 90 patients intubated in ICU were enrolled in this study. Patients were divided into VAP group (n = 45), non-VAP group (n = 45). The HRG, sTREM-1, C reactive protein (CRP) and white blood cells levels were measured on admission & 72 hours after intubation. **Results:** The study found that patients with ventilator-associated pneumonia (VAP) have a unique and deteriorating biomarker profile over time. Compared to non-VAP patients, VAP patients had significantly lower HRG levels and higher sTREM-1 levels. Initially, VAP patients had low HRG and moderate sTREM-1 levels, but over 72 hours, HRG levels decreased further while sTREM-1 levels increased, indicating a worsening inflammatory response. **Conclusion:** sTREM-1 levels at 72 hours were a stronger predictor of VAP than HRG levels**,** monitoring HRG and sTREM-1 levels over time could help doctors diagnose ventilator-associated pneumonia (VAP) earlier, identify patients at higher risk, and tailor treatments to individual needs.

Introduction

Ventilator-associated pneumonia (VAP) is a prevalent and potentially life-threatening hospital-acquired infection, particularly affecting intubated patients in intensive care units (ICUs). Despite various preventive measures, VAP remains a significant complication, with a rising incidence of multidrug-resistant organisms, which increases mortality rates. Early diagnosis, accurate classification, and prognosis estimation are crucial for improving clinical outcomes [1]. The Acute Physiology and Chronic Health Evaluation (APACHE) scoring system is used to assess illness severity and predict outcomes in Ventilator-Associated Pneumonia patients. Higher APACHE scores indicate more severe illness and greater risk of complications or mortality, considering physiological parameters, age, and chronic health

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conditions. Elevated APACHE scores in VAP patients correlate with increased risk of infection, prolonged mechanical ventilation, longer ICU stays, and higher mortality rates, guiding clinicians in treatment decisions and prognosis [2]. Several biomarkers, including C reactive protein (CRP), procalcitonin (PCT), and endotoxin, have been explored for diagnosing and assessing VAP severity. However, existing biomarkers have limitations, such as low specificity and restricted applicability to certain bacterial types, highlighting the need for developing and validating new biomarkers to enhance VAP diagnosis and management [3]. Histidine-rich glycoprotein (HRG) is a multi-domain protein produced by the liver that plays a crucial role in regulating various physiological processes, including immune responses, vascular function, fibrinolysis, and coagulation [4]. Additionally, HRG has been implicated in several pathological processes and diseases, such as inflammation, cancer, and sepsis, highlighting its potential as a biomarker or therapeutic target for these conditions [5].

Triggering Receptor Expressed on Myeloid cells-1 (TREM-1) is an innate immune receptor expressed on immune cells including: neutrophils, monocytes/macrophages, and endothelial cells, existing in two forms: membrane-bound Triggering Receptor Expressed on Myeloid cells-1 (mTREM-1) and Soluble Triggering Receptor Expressed on Myeloid cells-1 (sTREM-1) [6]. The mTREM-1 structure consists of three domains: an Ig-like domain responsible for binding ligands, a transmembrane domain that anchors the protein, and a cytoplasmic domain that associates with the DAP12 protein, and upon activation [7], it triggers a pro-inflammatory response, increasing pro-inflammatory cytokines like IL-6, IL-8, IL-1β, and TNF-α, promoting cell survival, and blocking anti-inflammatory cytokines like IL-10 [8-11]. sTREM-1 can be generated through proteolytic cleavage of mTREM-1 or alternative splicing of TREM-1 mRNA. sTREM-1 negatively regulates mTREM-1 signaling by neutralizing its ligands. Elevated levels of sTREM-1 in body fluids have been linked to poor clinical outcomes in various inflammatory conditions, including infectious and non-infectious diseases [12-14].

This study aimed to investigate and compare the diagnostic and prognostic value of sTREM-1and HRG in adult ICU patients with ventilator-associated pneumonia, addressing a current knowledge gap in this area.

Research Design and Methods

Study Population and Setting: This case-control study recruited participants from the Intensive Care Unit (ICU) department of Suez Canal University Hospital, collecting demographic characteristics from all participants. Additional data, including patient type (medical or surgical) and disease severity (APACHE score at admission), were obtained from the hospital's electronic medical record system. Laboratory analyses were conducted in the Clinical Pathology department. The study included two groups of 45 patients each: one group with VAP and another group without VAP, all admitted to the ICU. To participate, patients must be adults over 18, intubated with mechanical ventilation, and provide informed consent. Patients with any condition that causes inflammation and could alter the levels of the biomarkers being measured were excluded from the study such as: severe illness, tumors, infections, autoimmune diseases, pregnancy, or organ dysfunction

Sample size: The sample size calculation utilized a formula established by Dawson and Trapp [15], incorporating the following parameters:

$$n = 2\left[\frac{\left(Z_{\alpha/2} + Z_{\beta}\right) * \sigma}{\mu_1 - \mu_2}\right]^2$$

- Sample size (n)
- Z α/2 = 1.96 (95% confidence interval)
- Zβ = 0.84 (80% power)
- Standard deviation (σ) = 7.5 μg/mL
- Mean HRG levels: μ1 = 20.97 μg/mL (severe CAP group) and μ2 = 91.31 μg/mL (healthy group) [16].

Using this formula, the final sample size was 45 patients per group.

Sampling technique: A probability sampling approach was employed to randomly select 45 patients from each group within the adult ICU and emergency unit populations. Patients in both groups underwent comprehensive evaluations, received standard care, and were subjected to laboratory investigations, including hematological and microbiological analyses. Blood specimens were collected at predetermined timepoints (0 and 72 hours post-mechanical ventilation) and subsequently analyzed for inflammatory biomarkers, specifically CRP, HRG, and sTREM-1,

utilizing enzyme-linked HRG were promptly centrifuged at 3500 rpm for 10 minutes, and the resulting supernatant was collected and frozen at -80°C. The levels of serum HRG and sTREM-1were determined using ELISA kits from My BioSource (MBS2516267) and BT LAB (E6856Hu), respectively, following the manufacturers' protocols. In contrast, CRP levels were quantified using a fully automated Cobas c 6000 auto-analyzer from Roche Diagnostics (Mannheim, Germany) within the hospital's laboratory.

Data management: Statistical analysis was conducted using SPSS version 22.0 for Windows. Descriptive statistics were presented as mean ± standard deviation (SD) or percentages. Categorical variables were analyzed using Fisher's exact test or chi-square test, as applicable. Differences in means between groups were assessed using independent t-tests or Mann-Whitney U tests, depending on data normality. Regression analysis was performed to investigate the impact of patient baseline characteristics on study outcomes. A p-value < 0.05 was considered statistically significant for all tests.

Ethical consideration: This study was conducted in accordance with the ethical guidelines set by the Ethics Committee of Suez Canal University Faculty of Medicine, as evidenced by approval number 5890#. To ensure participant protection, written informed consent was obtained from each participant (or their legal guardian) prior to initiating data collection and conducting investigations.

Results

Table (1) presents demographic characteristics of 90 ICU patients, comparing 45 with VAP to 45 without VAP. The VAP group was significantly younger (46.42±9.65 years) than the non-VAP group (52.09±7.62 years, p=0.003). The majority of VAP patients were surgical (73.3%), differing significantly from non-VAP patients (33.3%, p=0.001). No significant differences were observed in sex, BMI, or smoking status between the two groups.

As presented in **Table 2,** the VAP group demonstrated significant changes in several biomarkers after 72 hours, compared to the non-VAP group. Specifically, there were significant increases in sTREM-1, white blood cell (WBC) count, and CRP levels. Conversely, a significant decrease was observed in HRG in VAP patients at 72 hours after admission.

Table 3 found no significant difference between the two groups regarding the presence of infection, as evidenced by non-significant differences in sputum culture and blood culture results. However, a significant difference was observed in the high-risk Acute Physiology and Chronic Health Evaluation (APACHE) score between the two groups, indicating a more severe disease state in the VAP group.

Figure (1-3) and table (4) present the results of a receiver operating characteristic (ROC) analysis, assessing the diagnostic efficacy of various biomarkers for VAP. The markers' performance on admission shows varying levels of predictive value for VAP. CRP (mg/dl) on admission stands out with an AUC of 0.989, sensitivity of 93.3%, and specificity of 97.8%, indicating excellent diagnostic accuracy. HRG on admission and WBCs on admission also show good predictive value, with AUCs of 0.944 and 0.896, respectively. In contrast, sTREM-1 on admission has a lower AUC of 0.631, indicating a relatively poor predictive value. After 72 hours, the levels of various markers were reassessed for their predictive value for VAP. Notably, sTREM-1 after 72 hrs emerged as a top performer, with an AUC of 0.980, sensitivity of 95.6%, and specificity of 97.8%, indicating excellent diagnostic accuracy. HRG µg/dl after 72 hrs also showed high predictive value, with an AUC of 0.974, sensitivity of 97.8%, and specificity of 93.3%. In contrast, CRP (mg/dl) after 72 h and WBCs after 72 h had relatively lower AUCs of 0.844 and 0. 697, respectively, indicating decreased predictive value over time.

The binary logistic regression analysis in **Table 5** identified significant predictors of Ventilator-Associated Pneumonia, including WBCS at admission (OR: 0.36), WBCS 72h after admission (OR: 1.45), HRG at admission (OR: 1.15), HRG 72h after admission (OR: 0.73), and sTREM-1 72h after admission (OR: 1.10). These biomarkers showed varying associations with VAP risk at admission and 72 hours after admission, while CRP, sTREM-1 at admission, and APACHE score were not significant predictors.

This correlation analysis in **figure 4** and **table 6,** examines the relationship between HRG levels at admission and 72 hours after admission, and various other variables. Key findings include negative correlations between HRG on admission and WBCS/CRP on admission, indicating higher HRG levels are associated with lower WBCS and

CRP levels. HRG on admission also showed a positive correlation with sTREM-1 on admission. In contrast, HRG 72 hours after admission was positively correlated with WBCS and CRP 72 hours after admission but negatively correlated with sTREM-1 72 hours after admission. Notably, APACHE SCORE showed no significant correlations with HRG levels at admission or 72 hours after admission.

In figure 5 and table 7 sTREM-1 on admission is negatively correlated with WBCS (r = -0.208, p = 0.049) and CRP (r = -0.182, p = 0.086). In contrast, sTREM-1 72 hours after admission is positively correlated with WBCS 72 hours after admission (r = 0.399, p = 0.001), CRP 72 hours after admission (r = 0.534, p = 0.001), and APACHE SCORE (r = 0.213, p = 0.04), while being negatively correlated with WBCS on admission (r = -0.602, p = 0.001) and CRP on admission (r = -0.724, p = 0.001).

The scatter plot matrix in **figure 6** revealed an inverse relationship between HRG and sTREM-1, with higher HRG levels associated with lower sTREM-1 levels, and vice versa, also there is possible stability or trends in HRG and sTREM-1 changes over time, with clustering of data points at admission and 72 hours later.

The box-and-whisker plot shows that VAP patients have lower HRG levels and higher sTREM-1 levels compared to non-VAP patients. Over 72 hours, HRG decreases further in VAP patients, while sTREM-1 increases. In contrast, non-VAP patients maintain higher HRG and lower sTREM-1 levels.

Table 1. Demographic characters between studied groups

	VAP N=45	Non VAP N=45	Test of significance
Age / years	46.42±9.65	52.09±7.62	t=3.09 p=0.003*
Sex Male Female	 32(71.1) 13(28.9)	 28(62.2) 17(37.8)	 χ^2=0.800 P=0.371
BMI (Kg/m^2)	25.99±4.55	25.64±4.09	t=0.377 p=0.707
Smoking -VE +VE	 14(31.1) 31(68.9)	 17(37.8) 28(62.2)	 χ^2=0.443 P=0.658
Type of patient Surgical Medical	 33(73.3) 12(26.7)	 15(33.3) 30(66.7)	 χ^2=14.46 P=0.001*

t: Student t test , χ^2= Chi-Square test , *statistically significant

Table 2. Comparison of inflammatory markers between studied groups

		VAP N=45	Non-VAP N=45	Test of significance
WBCS	On admission	5(4.25-5.85)	12(11-13)	Z=6.48 P=0.001*
	72h after admission	21(17-23.5)	17(15-22)	Z=3.23 P=0.001*
P value#		<0.001*	<0.001*	
CRP (mg/dl)	On admission	1(0.4-1.5)	7.3(4.9-10.3)	Z=8.0 P=0.001*
	72h after admission	17(14-22)	12(9-13.75)	Z=5.63 P=0.001*
P value#		<0.001*	<0.001*	
HRG (µg/dl)	On admission	89(69.5-113.5)	57(44.5-60)	Z=7.27 P=0.001*
	72h after admission	9(5.5-12.25)	41(29.5-45)	Z=7.74 P=0.001*
P value#		<0.001*	<0.001*	
sTREM-1(pg/ml)	On admission	175.67±17.68	163.89±25.95	t=2.52 p=0.014*
	72h after admission	322.24±22.91	236.76±31.58	t=14.69 p=0.001*
P value#		<0.001*	<0.001*	

*Data expressed as mean ±SD , Median (IQR) , *statistically significant , t :Student t test , Z:Mann Whitney U test ,#used test : Wilcoxon signed Rank test and Paired t test*

Table 3. comparison of sputum, blood culture, APACHE score between studied groups

	VAP N=45(%)	Non-VAP N=45(%)	Test of significance
Sputum culture (+VE)	35(77.8)	27(60)	χ^2=3.32 P=0.069
Blood culture(+VE)	29(64.4)	29(64.4)	P=1.0
APACHE SCORE Mean ±SD	24.82±3.09	23.49±4.21	t=1.71 p=0.09
Moderate risk High risk Very high risk	3(6.7) 41(91.1) 1(2.2)	13(28.9) 30(66.7) 2(4.4)	χ^2=8.28 P=0.016*

t: Student t test , χ^2= Chi-Square test , *statistically significant

Table 4. ROC curve showing validity of inflammatory markers between the studied groups

Test Result Variable(s)	Area	Std. Error[a]	P value	Asymptotic 95% Confidence Interval		Cut of point	Sensitivity (%)	Specificity (%)
				Lower Bound	Upper Bound			
HRG on admission	.944	.022	.001*	.902	.987	≥68	82.2	91.1
HRG µg/dl after 72 hrs	.974	.018	.001*	.939	1.000	≤22.5	97.8	93.3
sTREM-1 on admission	.631	.059	.032*	.515	.747	≥167.5	75.6	46.7
sTREM-1 after 72 hrs	.980	.019	.001*	.944	1.000	≥299.5	95.6	97.8
CRP (mg/dl) on admission	.989	.009	.001*	.972	1.000	≤2.6	93.3	97.8
CRP (mg/dl) after 72 h	.844	.041	.001*	.763	.925	≥13.75	80.0	75.6
WBCs on admission	.896	.044	.001*	.810	.982	≤9.25	97.8	88.9
WBCs after 72 h	.697	.055	.001*	.590	.804	≥17.5	73.3	51.1

*statistically significant

Table 5. binary logistic regression for predictors of VAP among studied cases

Predictor (s)	B	SE	P value	Odds ratio (95%CI)
WBCS On admission	-1.022	.227	.001*	.360(0.231-0.561)
WBCS 72h after admission	.372	.102	.001*	1.451(1.18-1.77)
CRP (mg/dl) On admission	-13.275	1154.476	.991	Undefined
CRP (mg/dl)72h after admission	3.693	581.653	.995	Undefined
HRG (µg/dl)On admission	.140	.058	.016*	1.15 (1.03-1.28)
HRG (µg/dl)72h after admission	-.311	.110	.005*	.732(0.591-0.908)
sTREM-1(pg/ml)On admission	-.029	.025	.242	.971(0.925-1.02)
sTREM-1(pg/ml)72h after admission	.098	.024	.001*	1.103(1.05-1.16)
APACHE SCORE	0.11	0.059	0.093	1.11(0.984-1.24)

*β : regression coefficient , SE: Standard error , *statistically significant*

Table 6. correlation between HRG (µg/dl) and all other inflammatory markers among studied cases

	HRG (µg/dl)			
	On admission		72h after admission	
	R	*P*	*R*	*P*
WBCS On admission	-0.608	0.001*	0.546	0.001*
WBCS 72h after admission	0.315	0.002*	-0.243	0.021*
CRP (mg/dl)On admission	-0.706	0.001*	0.669	0.001*
CRP (mg/dl)72h after admission	0.346	0.001*	-0.564	0.001*
sTREM-1(pg/ml)On admission	0.200	0.059	-0.243	0.021*
sTREM-1(pg/ml)72h after admission	0.708	0.001*	-0.705	0.001*
APACHE SCORE	0.125	0.240	-0.151	0.154

*r:Spearman Correlation coefficient *statistically significant*

Table 7. correlation between sTREM-1(pg/ml) and all other inflammatory markers among studied cases

	sTREM-1(pg/ml)			
	On admission		72h after admission	
	R	*P*	*R*	*P*
WBCS On admission	-0.208	0.049*	-0.602	0.001*
WBCS 72h after admission	-0.003	0.976	0.399	0.001*
CRP (mg/dl) On admission	-0.182	0.086	-0.724	0.001*
CRP (mg/dl)72h after admission	0.140	0.189	0.534	0.001*
APACHE SCORE	0.122	0.251	0.213	0.04*

*r: Spearman Correlation coefficient *statistically significant*

Figure 1. ROC curve showing CRP, HRG and STREM-1 on admission and after 72 hours.

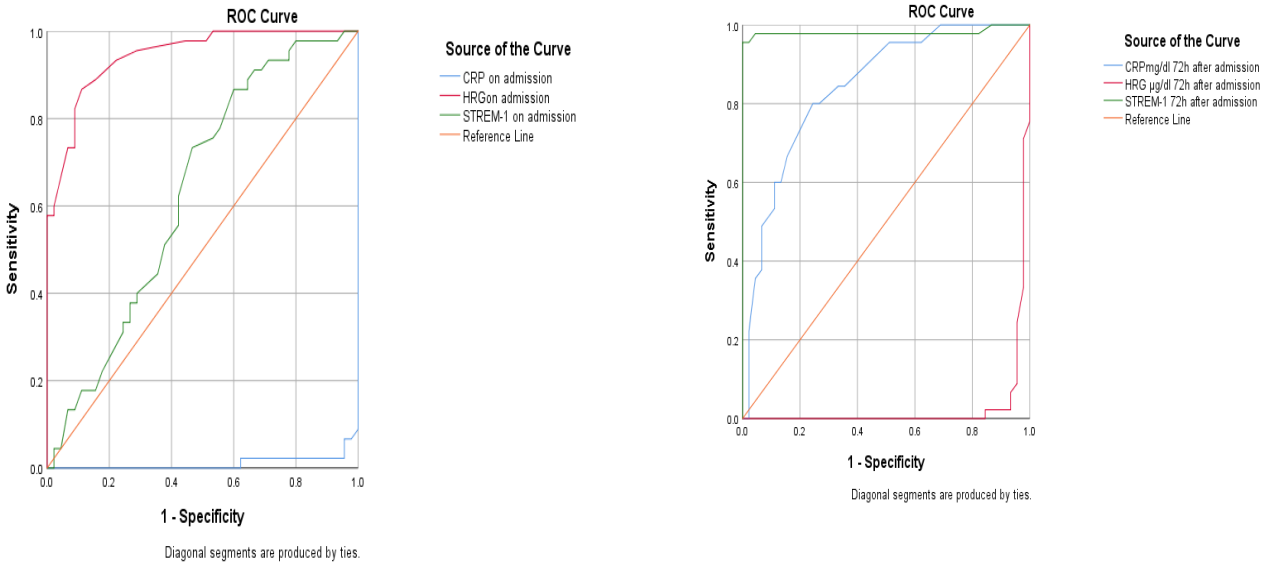


Figure 2. ROC curve showing CRP, WBCS admission. **Figure 3.** ROC curve showing CRP, WBCS after 72 hours of admission**.**

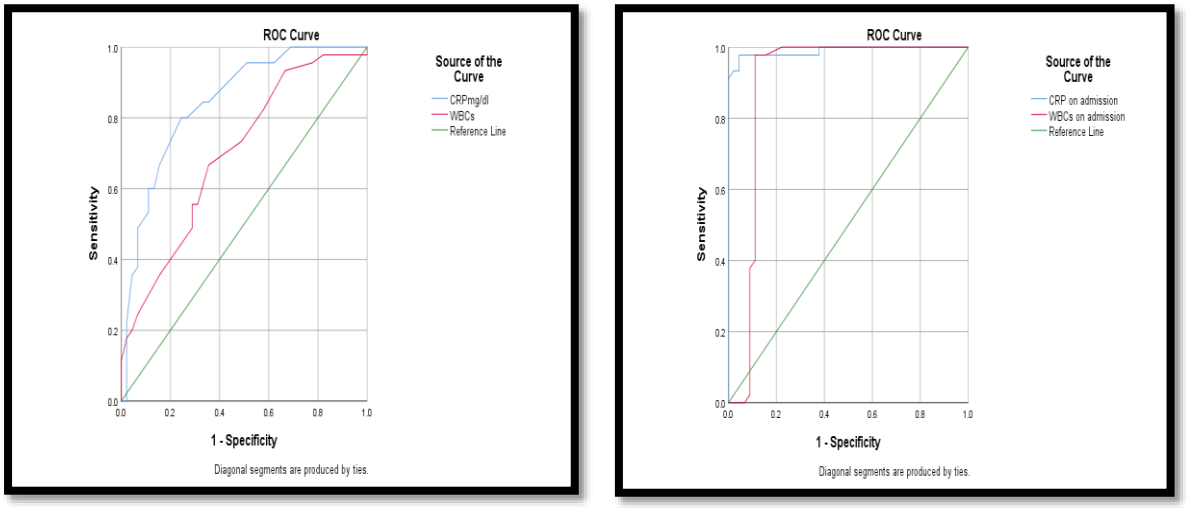


Figure 4. Scatter diagram showing correlation between HRG and all other assessed inflammatory markers.

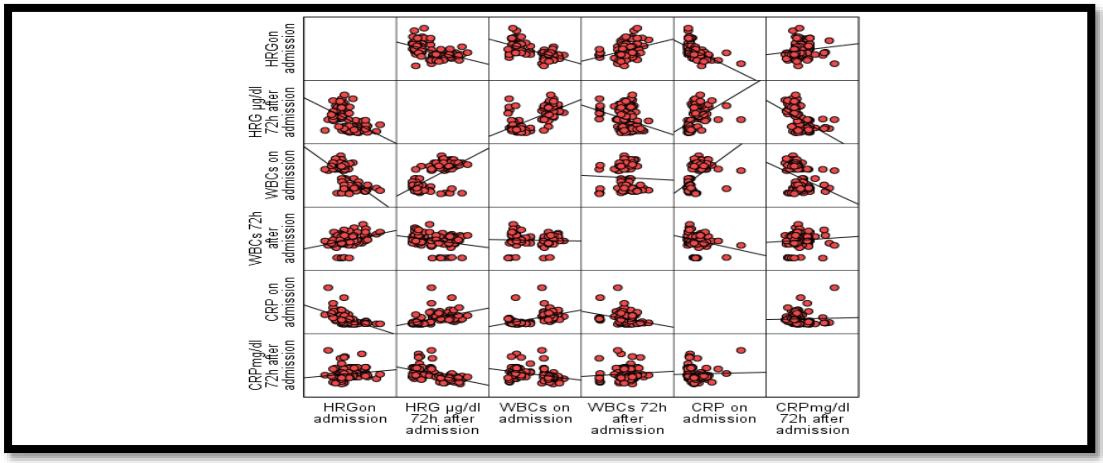


Figure 5. Scatter diagram showing correlation between STERM-1 and all other assessed inflammatory markers.

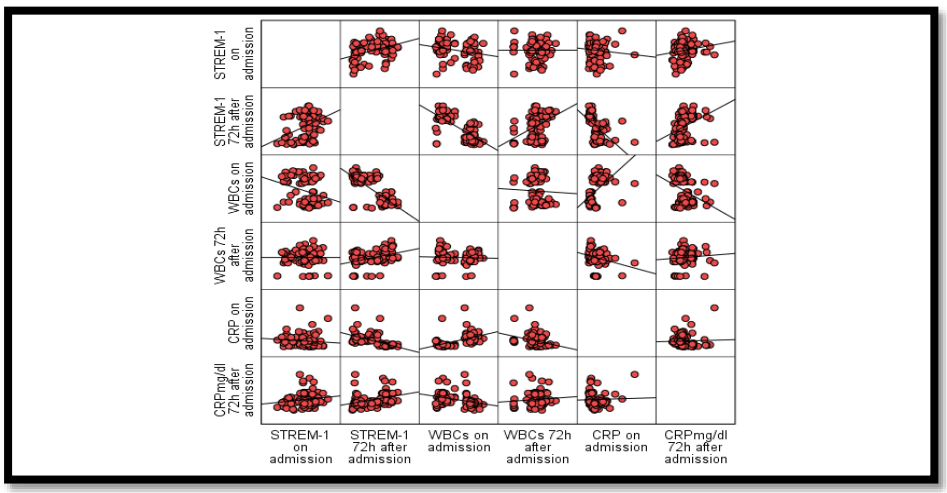


Figure 6. Scatter diagram showing correlation between STERM-1 and HRG.

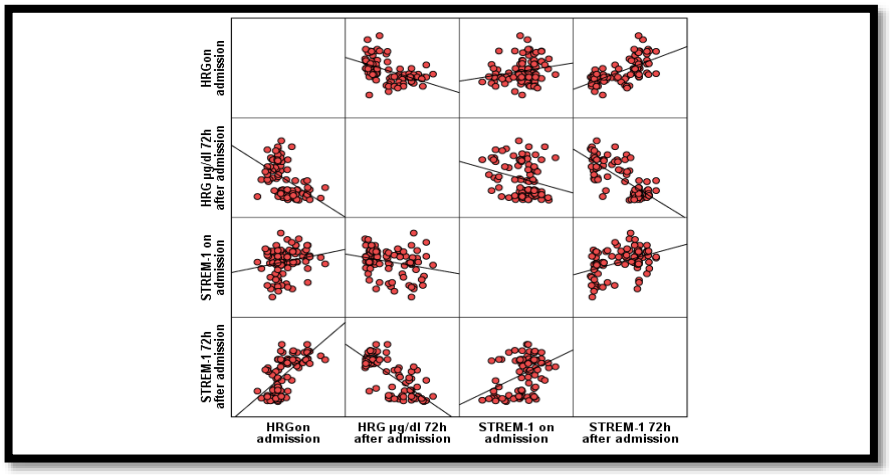
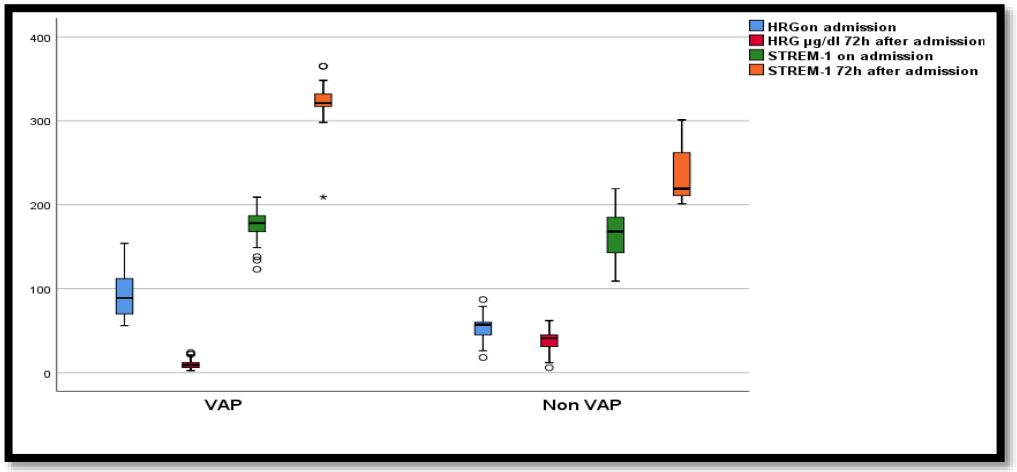


Figure 7. Box and Whisker plot showing median HRG and STERM-1 on admission and after 72 hours between VAP versus non-VAP group.



Discussion

Our study of 90 ICU patients compared 45 with VAP to 45 without VAP. Our finding that patients with VAP were significantly younger (46.42 ± 9.65 years) than those without VAP contrasts with studies suggesting advanced age as a VAP risk factor [17]. However, other research indicates age may not be a significant risk factor, with similar VAP rates across age groups [18]. The discrepancy may be due to differences in patient populations, underlying health conditions, or ICU practices. Our study also found a significantly higher proportion of surgical patients in the VAP group (73.3%) compared to the non-VAP group (33.3%, p=0.001). This supports existing literature identifying recent surgery as a significant risk factor for VAP [19], likely due to factors like prolonged mechanical ventilation, invasive procedures, and surgical stress response. Our study found no significant differences in sex, BMI, or smoking status between VAP and non-VAP groups. This contrasts with some studies identifying male sex as a VAP risk factor [18], while others report mixed findings regarding BMI and smoking status. The discrepancies highlight the complexity of VAP risk factors, emphasizing the need for further research with diverse populations to inform effective prevention and management strategies.

Biomarker changes in ventilator-associated pneumonia patients provide valuable insights into the pathophysiology and potential diagnostic markers of VAP. Compared to the non-VAP group, the VAP group exhibited significant alterations in biomarker levels at 72 hours, including elevated sTREM-1, WBC count, and CRP levels, as well as decreased HRG levels. sTREM-1 is a recognized biomarker for bacterial infections and sepsis, amplifying the immune response, Research has shown that elevated sTREM-1 levels are associated with ventilator-associated pneumonia. A study by

Zhao et al. found that sTREM-1 is a reliable predictor of VAP in neonates, particularly when combined with PCT measurements after 72 hours of mechanical ventilation, providing the highest predictive accuracy for VAP [20]. **Fernando et al.** observed that although elevated WBC counts were prevalent in VAP patients, this biomarker lacked specificity, as it failed to reliably differentiate VAP from other inflammatory conditions [21]. CRP is a well-established biomarker of inflammation. The findings of **Povoa et al.**, which reported elevated CRP levels in VAP patients, support the utility of CRP as a complementary diagnostic tool, particularly when used in conjunction with other biomarkers for the diagnosis of VAP [22]. CRP is a well-established inflammatory marker. **Wang et al.** also found higher CRP levels in VAP patients, supporting its use in conjunction with other biomarkers for VAP diagnosis [23]. **Pradana et al**. demonstrated that decreased HRG levels are associated with immune dysregulation and may have prognostic value as a biomarker in ICU patients with infections, suggesting its potential utility in predicting clinical outcomes [24]. These biomarkers, individually and in combination, may enhance the early diagnosis and management of VAP.

Notably, our study revealed that the presence of infection, as indicated by sputum and blood culture results, did not significantly differ between the VAP and non-VAP groups. However, a significant distinction was observed in the APACHE scores, with the VAP group exhibiting higher scores, suggesting a more severe clinical presentation and disease state in VAP patients. Sputum cultures for VAP diagnosis are contentious, as while they can detect potential pathogens, their accuracy is compromised by colonization. Furthermore, culture results may be reported in various formats, necessitating cautious interpretation to determine clinical significance [25]. Blood cultures can play a valuable role in VAP diagnosis, particularly when respiratory cultures yield inconclusive results. Additionally, they can help clinicians detect concurrent infections outside the respiratory tract [26]. The APACHE II score is a valuable predictor of outcomes in VAP patients. Research has shown that higher APACHE II scores at diagnosis are associated with increased mortality, with significantly higher scores observed in non-survivors compared to survivors suggesting its utility in predicting outcomes in VAP patients [27]. While other scoring systems, such as SOFA and CPIS, have been explored, research suggests that APACHE II may have superior predictive ability for mortality in VAP patients [28]. Our study supports existing research indicating that traditional infection markers, such as sputum and blood cultures, may not reliably distinguish between VAP and non-VAP patients due to limitations like colonization and low sensitivity. The significant difference in APACHE scores highlights the importance of assessing disease severity, as higher scores are associated with poorer outcomes in VAP patients. These findings emphasize the need for comprehensive clinical evaluation and the potential integration of multiple diagnostic tools and scoring systems to accurately identify and manage VAP.

Our finding that CRP is a reliable early indicator of VAP is supported by previous research. CRP levels rise in response to inflammation, making it a useful marker for infections like VAP [22]. However, CRP can also be elevated in non-infectious conditions, limiting its specificity [29], therefore, CRP should be considered alongside other clinical factors to accurately diagnose VAP. **Our study shows that sTREM-1 becomes a top performer for diagnosing VAP after 72 hours,** which is interesting because earlier research had mixed results. A study found that sTREM-1 levels were higher in neonates who developed VAP after 72 hours of mechanical ventilation. The study showed that measuring sTREM-1 after 72 hours was a reliable way to predict VAP, with high sensitivity and specificity [20], while others found it less useful, possibly due to factors like prior antibiotic use and variations in study design [30]. These findings highlight that sTREM-1 levels change over time, making it a potentially useful tool for diagnosing VAP at the right moment. As infection worsens, sTREM-1's role in the inflammatory response becomes more significant, increasing its diagnostic value. Considering the timing of sTREM-1 measurement in clinical protocols could lead to more accurate VAP diagnoses and better patient outcomes [31]. Our findings on HRG as a strong predictor of Ventilator-Associated Pneumonia are significant. Recent studies have explored HRG's role in infectious and inflammatory conditions, providing context for our results. Two studies, one by Ding et al. and another by He et al., support HRG's prognostic value. They found that lower HRG levels were associated with worse outcomes, including increased disease severity and higher

mortality rates, in patients with VAP and community-acquired pneumonia (CAP) [32,16]. Another study **by Oiwa et al.** found that lower HRG levels in postoperative ICU patients were associated with more complications [33]. sTREM-1 is a better predictor of VAP because its levels increase rapidly and sharply in response to bacterial infections. This makes it a sensitive indicator of the severity of the infection. In contrast, HRG is an anti-inflammatory protein that decreases more slowly and is influenced by other factors. This makes it less directly related to the acute inflammatory response seen in VAP, Therefore, sTREM-1 is a stronger predictor of VAP because it more closely reflects the evolving inflammatory state of the infection [34,35].

In our study the WBC count at admission has an odds ratio (OR) of 0.36, suggesting a protective effect, while WBC count 72 hours post-admission shows an OR of 1.45, indicating an increased risk of VAP. This temporal shift underscores the dynamic inflammatory response in VAP patients. A study by Seligman et al. found that decreases in PCT and CRP are strong predictors of survival in VAP. Monitoring inflammatory markers like WBCs, PCT, and CRP can provide valuable prognostic information in VAP patients. A decreasing trend in these markers indicates a favorable response to treatment and better prognosis, while increasing levels signal worsening infection or complications. Regular assessments of these biomarkers can enhance early detection and allow for timely interventions, potentially improving patient outcomes [36]. HRG exhibits a unique temporal relationship with VAP risk, with higher levels at admission associated with a modest increase in risk (OR: 1.15), while lower levels 72 hours later confer a protective effect (OR: 0.73), highlighting HRG's potential role in immune modulation and its promise as a diagnostic biomarker for VAP. Ding et al. (2018) reported that serum HRG levels decrease in patients developing VAP, supporting its potential as a diagnostic biomarker [32]. Our finding that an OR of 1.10 for sTREM-1 at 72 hours post-admission is linked to increase in VAP risk is consistent with previous research, which suggests that elevated sTREM-1 levels are associated with VAP, making it a promising biomarker for early VAP diagnosis. Research on sTREM-1 as a VAP biomarker yields mixed results. **Zhao et al.** found sTREM-1 levels were higher in neonates with VAP after 72 hours of ventilation, with high sensitivity and specificity [20], however, **Shirani and Hajzargarbashi** reported no significant difference in sTREM-1 levels between VAP and non-VAP groups, suggesting CRP and PCT may be more reliable markers [35]. These discrepancies highlight the importance of considering study design, patient population, and timing of biomarker measurement, and support the use of serial measurements and multi-biomarker approaches to improve VAP diagnosis. Our study found that CRP and APACHE II scores were not reliable predictors of VAP outcomes, which diverge from previous research, that suggests that CRP alone may not reliably predict VAP outcomes, whereas PCT levels may be more predictive [37]. **Melsen et al.,** additionally found that the APACHE II score at the time of VAP diagnosis may be useful in predicting mortality in ICU patients, highlighting the complexity of predicting VAP outcomes [38].

The correlation analysis reveals that HRG plays a dynamic role in VAP patients. Initially, high HRG levels correlate with low WBCs and CRP, suggesting anti-inflammatory effects, and positively with sTREM-1, indicating a coordinated immune response. However, 72 hours post-admission, HRG levels correlate positively with WBCs and CRP, reflecting a reactive response to ongoing inflammation, and negatively with sTREM-1, implying a transition to a regulated immune response. Notably, HRG levels do not correlate with APACHE II scores, suggesting HRG's potential as an independent biomarker for tracking host response in VAP. Recent studies support the protective role of HRG in critically ill patients. Ding et al, found lower HRG levels were associated with poorer outcomes in VAP patients [32]. Oiwa et al. linked decreased HRG levels to a higher incidence of complications in postoperative ICU patients [33]. Kawanoue et al. discovered consistently low HRG levels were a strong predictor of mortality in septic patients, highlighting HRG's prognostic value beyond traditional severity scores [39].

Our results demonstrate that sTREM-1 exhibits distinct temporal patterns in VAP patients. Initially, lower sTREM-1 levels correlate with higher baseline inflammatory markers, but as the disease progresses, increased sTREM-1 levels correlate positively with markers of inflammation and disease severity. These findings, which align with recent studies [6, 30] underscore the importance of serial biomarker measurements in guiding the management of VAP. Dynamic

monitoring of sTREM-1 through serial measurements can provide a more comprehensive understanding of a patient's inflammatory status in VAP. Combining sTREM-1 with other biomarkers and clinical severity scores can improve diagnosis and prognostication accuracy. This integrated approach may enable tailored interventions and potentially inform targeted therapies to modulate the inflammatory response and improve outcomes in VAP patients [35,40].

The box-and-whisker plot highlights distinct differences in biomarker levels between VAP and non-VAP patients. VAP patients showed lower HRG levels, indicating a diminished protective effect and potentially contributing to a more severe inflammatory response. In contrast, sTREM-1 levels were elevated in VAP patients, reflecting a heightened state of immune activation. These findings align with previous studies, suggesting that HRG and sTREM-1 may serve as valuable biomarkers for diagnosing and monitoring VAP [32,33,35,41].

Conclusions: The study concludes that patients with VAP show a distinct and worsening biomarker profile over time, with significantly lower HRG levels and higher sTREM-1 levels compared to non-VAP patients. Initially, VAP patients exhibit lower HRG and modest sTREM-1 levels, but over 72 hours, HRG further declines while sTREM-1 increases, indicating an escalating inflammatory response. Notably, sTREM-1 is more valuable than HRG levels at 72 hours after admission as a strong predictor of VAP. These dynamic changes, along with altered correlations between HRG, sTREM-1, and inflammatory markers like WBCs and CRP, suggest that serial monitoring of these biomarkers could greatly enhance early diagnosis, risk stratification, and targeted treatment in VAP patients, also the need for further research to elucidate the underlying mechanisms.

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