



Корреляция между повышенным уровнем соотношения нейтрофилов и лимфоцитов, показателем вазотропности и инотропности, суммарным балансом жидкости и уровнем активных форм кислорода у пациентов с сепсисом

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Введение. Активные формы кислорода (ROS) служат биомаркером сепсиса, в то время как соотношение нейтрофилов и лимфоцитов (NLR), показатель вазотропно-инотропного действия (VIS) и общий баланс жидкости (CFB) коррелируют с воспалением и смертностью. Понимание корреляции между повышенным уровнем ROS и этими маркерами помогает в прогнозировании.

Цель – изучение корреляции между повышенным уровнем соотношения нейтрофилов и лимфоцитов, показателем вазотропности и инотропности, суммарным балансом жидкости и уровнем активных форм кислорода у пациентов с сепсисом.

Материалы и методы. В проспективном когортном исследовании, проведенном в отделении интенсивной терапии доктора Муварди в RSUD с июня по август 2023 г., использовали одномерный, двумерный (корреляция Спирмена) и многомерный логистический регрессионный анализы.

Результаты. У 34 пациентов с сепсисом выявлена значимая положительная корреляция показателей NLR и VIS в первый день, а показателей NLR, VIS и FB – на третий день. Дельта-изменения показателей NLR и VIS достоверно коррелировали с ROS.

Заключение. изменения NLR и VIS предсказывают уровни ROS, в то время как CFB демонстрирует слабую корреляцию, которая заслуживает дополнительного изучения. Это подчеркивает важность NLR и VIS как прогностических показателей при сепсисе.

Ключевые слова: соотношение нейтрофилов и лимфоцитов, NLR, показатель вазотропно-инотропного действия, VIS, суммарный баланс жидкости, CFB, активные формы кислорода, АФК, сепсис, септический шок

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Correlation between elevated neutrophil lymphocyte ratio, vasotropic inotropic score, cumulative fluid balance and level of reactive oxygen species in septic patients

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Introduction. Reactive oxygen species (ROS) serve as a biomarker in sepsis, while neutrophil lymphocyte ratio (NLR), vasotropic inotropic score (VIS), and cumulative fluid balance (CFB) correlate with inflammation and mortality. Understanding the correlation between elevated ROS levels and these markers aids in prognostication.

The objective is to study the correlation between the increased level of neutrophil/lymphocyte ratio, vasotropic and inotropic index, total fluid balance and reactive oxygen species level in patients with sepsis.

Materials and methods. A prospective cohort study in RSUD Dr. Moewardi's ICU/HCU from June to August 2023 employed univariate, bivariate (Spearman correlation), and multivariate logistic regression analyses.

Results. Among 34 sepsis patients, NLR and VIS showed significant positive correlations on day one, and NLR, VIS, and FB on day three. Delta changes in NLR and VIS significantly correlated with ROS.

Conclusion. NLR and VIS changes predict ROS levels, while CFB exhibits weak correlations, which deserve additional investigation. This underscores the importance of NLR and VIS as prognostic indicators in sepsis.

Key words: neutrophil lymphocyte ratio, NLR, vasotropic inotropic score, VIS, cumulative fluid balance, CFB, reactive oxygen species, ROS, sepsis, septic shock

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Introduction

The exact prevalence of sepsis in a country is frequently unclear. In 2017, research indicates that sepsis was a factor in approximately 33% to 50% of all hospital deaths in the United States. While the data reflects sepsis incidence in high-resource countries, the majority of sepsis-related deaths happen in low-resource countries, where correctly estimating the exact incidence of sepsis is challenging. Ap-

proximately 90% of sepsis-related deaths in cardiothoracic cases globally occur in low-resource nations. Around 70% of the 9 million deaths in neonates and babies are due to sepsis, with the bulk of cases happening in Asia, particularly Indonesia [1]. In 2017, the World Health Organization (WHO) adopted a resolution to enhance the prevention, detection, and management of sepsis. Objective risk stratification method has been utilized to identify organ dysfunction in patients at risk of sepsis. This

tool is used to determine the scores for sequential organ failure assessment (SOFA) and quick sequential organ failure assessment (qSOFA). Specific biomarkers such as C-reactive protein (CRP) and procalcitonin (PCT) have been linked to the likelihood of sepsis cases, as they are infection biomarkers. Both of these biomarkers are commonly used to detect sepsis as they can differentiate viral infections from bacterial infections. Reactive oxygen species (ROS) and neutrophil lymphocyte ratio (NLR) have been used to indicate cases of sepsis and septic shock [2].

Several studies have identified additional indicators that can serve as biomarkers in predicting cases of sepsis and septic shock. Recently, the NLR indicator derived from blood analysis has garnered attention in research on inflammation-related diseases. According to several studies, NLR can be used as a prognostic indicator for cancer and cardiovascular diseases. Studies on sepsis in adults indicate that NLR might be utilized as a biomarker to assess systemic inflammation [3]. Gaies et al. found that the vasoactive inotropic score (VIS) accurately represents cardiovascular dysfunction and predicts outcomes in infants after cardiopulmonary bypass. VIS is an indicator that calculates the total amount of cardiovascular support therapies such as dopamine, dobutamine, epinephrine, milrinone, vasopressin, and norepinephrine administered to patients [4]. Previous research has shown that VIS values correlate with poorer outcomes in infants after heart surgery. Other studies have shown that VIS is associated with cases of sepsis in pediatric patients [5]. J. Song et al. discovered that higher VISmax values within the initial 6 hours of patients arriving at the emergency department were linked to higher 30-day mortality rates in adult patients diagnosed with sepsis according to the Sepsis-3 criteria. VISmax was found to be superior to cardiovascular components in predicting death in sepsis patients within 30 days, as measured by the SOFA score and early lactate levels. It was also comparable to the acute physiology and chronic health evaluation (APACHE) II score in this regard [6].

Early therapy of sepsis should focus on promptly establishing vascular access and starting fluid resuscitation. Patients with sepsis should be given 30 mL of intravenous crystalloid per kilogram within the initial three hours. Excessive fluid retention is a predictor of mortality in patients with sepsis. Cumulative fluid balance (CFB) and sepsis-induced multi-organ dysfunction syndrome (MODS) are positively associated. The European SOAP project shown that the CFB 72 hours after septic shock onset correlates with the development of MODS and serves as a robust indicator of death in septic shock cases [7]. ROS has been utilized as a biomarker in sepsis and septic shock in emergency care for a considerable amount of time. Recent research have identified a correlation between NLR, VIS, and CFB levels in sepsis patients. However, no studies have explored the relationship between the increase in these markers and ROS levels in sepsis patients. Researchers aim to evaluate the relationship between elevated ROS levels and NLR, VIS, and CFB in sepsis patients.

Materials and methods

Study Design. This study is an analytical observational research conducted using a prospective cohort design. The target population in this study were all patients treated with sepsis in the Intensive Care Installation, in this case, the ICU and HCU at Dr. Moewardi from June 2023 – August 2023. The research sample is the entire population that meets the following criteria: Patients diagnosed with sepsis aged more than 18 years who are being treated in the ICU/HCU RSDM, and patients and their families are willing to participate in the research. The exclusion criteria were as follows: Patients with a history of using cytotoxic drugs, chemotherapy, or immunosuppressants, and patients with malignancies. Drop-out criteria are if the patient died before the third day of treatment. This research used a consecutive sampling technique. The sampling technique is non-random, namely selecting all subjects who meet the research criteria until the required sample size is met. This study employs written informed consent, where research participants or their families are provided with an explanation of the research and potential hazards. The research commenced following approval from the Research Ethics Commission of RSUD Dr. Moewardi and was carried out in accordance with the Declaration of Helsinki.

Data Collection. On the first and third day of sepsis diagnosis in the ICU/HCU ward, the patient's blood samples are taken, CFB data are documented, and information on the administration of inotropic and vasopressor medicines is gathered.

Neutrophil and lymphocyte levels are determined by analyzing a blood sample using a normal blood test. The neutrophil lymphocyte ratio (NLR) is calculated by dividing the number of neutrophils by the number of lymphocytes. Coding is done to categorize the NLR value into CFB groups.

CFB is assessed by quantifying the amount of fluid taken in and excreted during therapy and then determining the net balance. The equation for CFB is Input minus Output. When output exceeds input, it results in a negative CFB. Conversely, a positive CFB occurs when input surpasses output. A CFB of zero is achieved when output is equal to input.

The ROS level is determined by obtaining a blood sample and allowing it to coagulate for 10–20 minutes. The blood is centrifuged at the speed of 2000–3000 rotations per minute for 20 minutes to collect serum. The clinical laboratory department examines ROS levels using the ELISA technique. The ROS level is determined by measuring the optical density (OD) of each sample and comparing it to the microplate standard. Once the ROS level is acquired, coding is used to categorize it into high or low groups.

The VIS value is determined by the following formula: dopamine dose ($\mu\text{g/kg/minute}$) + dobutamine dose ($\mu\text{g/kg/minute}$) + $100 \times$ epinephrine dose ($\mu\text{g/kg/minute}$) + $10 \times$ milrinone dose ($\mu\text{g/kg/minute}$) + $10,000 \times$ vasopressin dose (unit/kg/minute) + $100 \times$ norepinephrine dose ($\mu\text{g/kg/minute}$).

Table 1. Basic characteristics of the subjects

Parameter	Mean $\pm$ SD	Median	Min	Maks	<i>p</i>
Age	52.79 $\pm$ 18.15	54.50	19.00	89.00	0.684
Gender <i>n</i> (%)					
Female	15 (44.1%)				
Male	19 (55.9%)				
Qsofa	2.79 $\pm$ 0.41	3,0	2,0	3,0	0
Pretest (H1)					
ROS	231.65 $\pm$ 96.46	230.50	90.00	412.00	0.011
NLR	18.00 $\pm$ 14.34	11.59	2.34	69.50	0
VIS	56.84 $\pm$ 118.77	15.00	5.0	440.0	0
CFB	353.00 $\pm$ 850.78	393.22	-1353.0	2110.0	0.763
Posttest (H3)					
ROS	237.88 $\pm$ 102.55	215.0	96.0	456.0	0.004
NLR	21.82 $\pm$ 18.16	17.54	3.25	68.79	0
VIS	62.65 $\pm$ 126.07	20.0	5.00	495.0	0
CFB	436.91 $\pm$ 1210.58	378.0	-2276.00	2922.19	0.578
Delta (difference)					
ROS	6.24 $\pm$ 79.82	-1.0	-142.00	201.0	0.045
NLR	3.83 $\pm$ 13.74	3.32	-19.40	54.27	0.007
VIS	5.81 $\pm$ 105.5	5.0	-390.00	455.0	0
CFB	83.91 $\pm$ 943.46	48.0	-1997.72	2541.55	0.788

Note: Shapiro–Wilk normality test, data are considered to meet the assumption of normality if the *p*-value is > 0.05 .

Data Analysis. We used tabular descriptions and characteristics of each research variable for univariate analysis. Categorical data are reported as frequency and percentage proportions meanwhile numerical data is presented as mean $\pm$ SD, median (min–max). The Shapiro–Wilk test is used to assess the normality of data. The *p*-value > 0.05 indicates normal distributed data. Pearson correlation is used for normally distributed data and Spearman correlation for non-normal data in bivariate analysis. A Pearson correlation value of 0 shows no association between two variables, +1 indicates a strong positive correlation and direction, and -1 indicates a strong negative correlation and direction. We employed linear regression analysis for multivariate analysis. It assesses the relationship between multiple independent variables and a dependent variable, predicting the dependent variable's value based on changes in the independent variables.

Results

Basic Characteristics. The study involved 34 sepsis patients from the ICU/HCU of Dr. Moewardi Hospital in Surakarta. The patients' ages ranged from 19 to 89 years, with a mean age of 52.79 ± 18.15 years. The majority of patients were male (55.9%), while the remaining were female (44.1%). Patients diagnosed with sepsis in the ICU/HCU had the average ROS score of 231.65 ± 96.46 on the first day (pretest) and 237.88 ± 102.55 on the third day (posttest). The average ROS rose by 6.24 ± 79.82 , equivalent to the 2.7% increase. The mean NLR score was 18.00 ± 14.34 on the initial day (pretest) and 21.82 ± 18.16 on the third day (posttest). The average

NLR delta increased by 21.3%, or 3.83 ± 13.74 . The mean VIS score was 56.84 ± 118.77 on the first day (pretest) and 62.65 ± 126.07 on the third day (posttest). The mean VIS rose by 10.2% to 5.81 ± 105.50 . The average CFB score was 353.00 ± 850.78 on the first day (pretest) and 436.91 ± 1210.58 on the third day (posttest). The mean value of CFB rose by 23.8% to 943.46 from 83.91. The complete description of the basic characteristics of the research data can be seen in the table 1 below.

According to the table 1, the normality test results for the ROS parameter in the pretest, posttest, and delta showed the *p*-values of 0.011, 0.004, and 0.045 ($p < 0.05$), respectively, suggesting that the data for the ROS parameter do not meet the normality assumption. The correlation analysis of all research parameters (NLR, VIS, and CFB) with the ROS parameter utilizes the non-parametric Spearman rank test.

Bivariate Analysis of Correlation Between Increase in NLR, VIS, CFB, and Increase in ROS in Sepsis Patients in the ICU/HCU. The correlation between the increase in NLR, VIS, CFB, and the increase in ROS in sepsis patients in the ICU/HCU can be seen in tables 2, 3, and 4 as follows.

The scatterplot in figures 1a, 1b, and 1c displays a linear trend from the bottom left to the top right, suggesting that as the NLR, VIS, and CFB values rise, the ROS examination results also increase. The table 2 indicates that on the initial day of diagnosing sepsis in the ICU/HCU, there is a significant positive association between NLR and ROS at the *p*-value of 0.009, falling within the moderate group of correlation strength ($r = 0.400–0.599$). VIS also has a positive and significant connection with ROS at the *p*-value of 0.006

Table 2. Correlation of NLR, VIS, and CFB with increased ROS on the first day in patients diagnosed with sepsis in the ICU/HCU

Parameter	Total Patients	NLR		VIS		CFB	
		<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>	<i>r</i>	<i>P</i>
ROS	34	0.442	0.009*	0.460	0.006*	0.119	0.504

Note: Spearman rank correlation test (for non-normally distributed numerical data). * – Significant at $p < 0.05$.

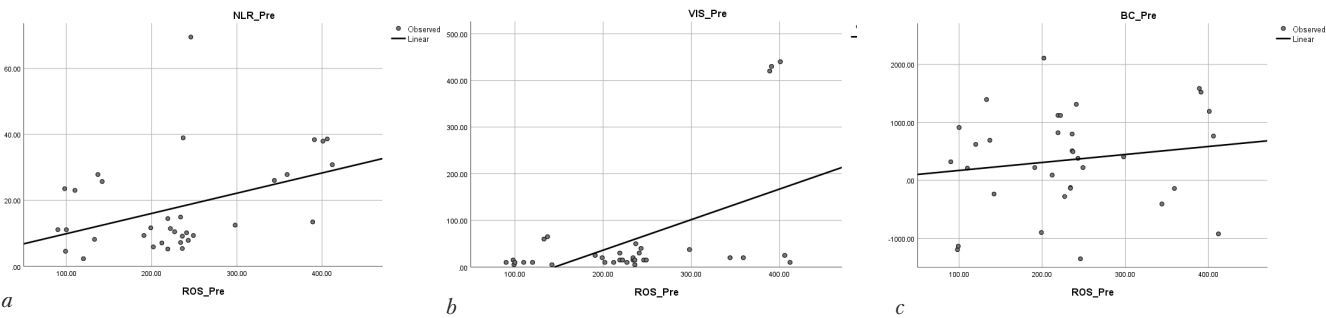


Fig. 1. Scatterplot of the first day correlation: a – NLR with ROS; b – VIS with ROS; c – CFB with ROS

Table 3. Correlation of NLR, VIS, and CFB with increased ROS on the third day in patients diagnosed with sepsis in the ICU/HCU

Parameter	Total Patients	NLR		VIS		CFB	
		<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>	<i>r</i>	<i>P</i>
ROS	34	0.451	0.007*	0.654	< 0.001*	0.399	0.019*

Note: Spearman rank correlation test (for non-normally distributed numerical data). * – Significant at $p < 0.05$.

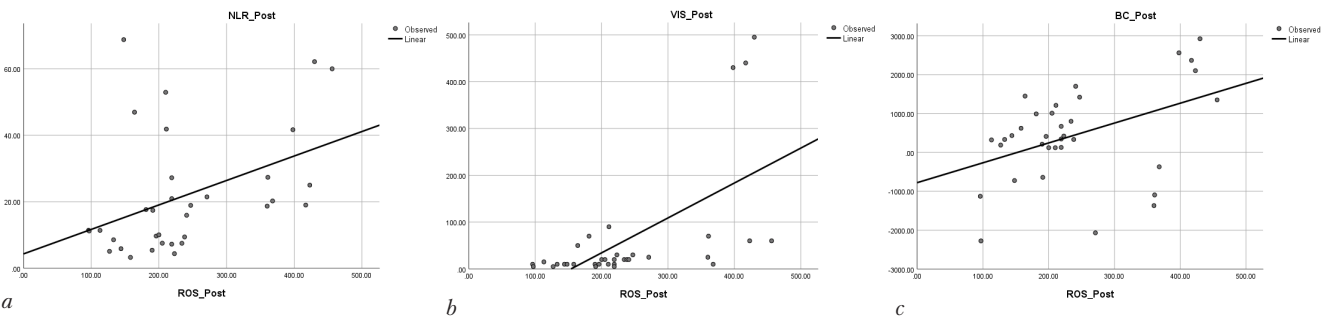


Fig. 2. Scatterplot of the third day correlation: a – NLR with ROS; b – VIS with ROS; c – CFB with ROS

Table 4. Multivariate analysis of variables correlated with delta changes in ROS increase in patients diagnosed with sepsis in the ICU/HCU

	Unstandardized Coefficients (B)	t	p-value
(Constant)	–3.377	–0.301	0.766
Delta NLR	2.060	2.249	0.032*
Delta VIS	0.298	2.496	0.018*
	$R^2 = 0.420$	$F = 11.209$	< 0.001*

Note: Linear Regression Analysis, * – significant at $p < 0.05$.

($r = 0.460$). The intensity of this correlation is within the moderate group ($r = 0.400–0.599$). Meanwhile, there is no significant association between CFB and ROS at the p -value greater than 0.05. The correlation strength is classified as very poor ($r = 0.000–0.199$). Thus, the increase in NLR and VIS may suggest a rise in ROS on the first day of sepsis diagnosis in the ICU/HCU, however the increase in CFB is not a reliable predictor of the increase in ROS on the first day of sepsis diagnosis.

The scatterplot graph in figures 2a, 2b, and 2c displays a linear trend from the bottom left to the top right, indicating that as NLR, VIS, and CFB examination findings rise, suggesting that as the NLR, VIS, and CFB values rise, the ROS examination results also increase. The table 3 shows a positive association between NLR and ROS on the third day of sepsis diagnosis in the ICU/HCU, with a moderate correlation strength ($r = 0.451$; $p = 0.007$). The correlation between VIS and ROS

is significant ($r = 0.654$; $p < 0.001$) and indicates a strong relationship ($r = 0.600$ – 0.799 ; $p < 0.05$). CFB ($r = 0.399$; $p = 0.019$) showed a positive and significant correlation with ROS at the p -value < 0.05 , with the strength of the correlation falling into the weak category ($r = 0.200$ – 0.399). Thus, the increase in NLR, VIS, and CFB may suggest a rise in ROS on the third day of sepsis diagnosis in the ICU/HCU.

Multivariate Analysis of Variables Correlated with Increased ROS Levels in Sepsis Patients in ICU/HCU. This study found a strong association between NLR and VIS delta change values and ROS rise in sepsis patients in the ICU/HCU when using multivariate analysis (Table 4). The table 5 shows multivariate analysis results.

Multivariate analysis showed an R^2 value of 0.420, indicating that NLR and VIS change can predict 42.0% of ROS rise, whereas 58.0% is influenced by other variables outside the research model. With the p -value of ≤ 0.001 , NLR and VIS increases correlate with contemporaneous ROS increases.

The delta NLR coefficient is 2.060, meaning ROS increases by 2.060 units for every unit increase in NLR. The p -value of 0.032 ($p < 0.05$) indicates a significant connection between raised NLR and elevated ROS. Any increase in VIS by 1 unit increases ROS by 0.298 units, according to the delta VIS coefficient. The p -value of 0.018 ($p < 0.05$) indicates a strong association between VIS and ROS increases.

Discussion

The study examined 34 sepsis patients diagnosed in the ICU/HCU of Dr. Moewardi Surakarta Hospital, ages ranging from 19 to 89 years, with the average age of 52.79 ± 18.15 . Overall, 55.9% of patients were male and 44.1% female. The mean qSOFA score was 2.79 ± 0.41 , ranging from 2 to 3. This study examined NLR, VIS, and CFB as ROS predictors, a crucial sepsis treatment metric. These biomarkers were measured on the first and third days of the trial, helping to understand sepsis dynamics. Sepsis arises from a dysregulation of the immune system that impacts inflammatory and anti-inflammatory processes. Enhanced control over the pro- and anti-inflammatory pathways results in the release of cytokines, mediators, and pathogen chemicals, activating coagulation and the complement cascade [8].

The NLR levels in sepsis patients increased from the average of 18.00 ± 14.34 on the first day to 21.82 ± 18.16 on the third day, indicating the 21.3% rise. This increase correlates with the rise in ROS, from the average of 231.65 ± 96.46 to 237.88 ± 102.55 , marking the 2.7% increase over the same period.

The results were supported by the study conducted by Velissaris et al., in which many research investigations demonstrated a direct relationship between the severity of disease in critically ill patients and the extent of neutrophilia and lymphocytopenia. In addition, four other meta-analyses have validated the trend of elevated NLR in patients with severe sepsis [9,10]. The

rise in both NLR and ROS from the initial day of sepsis diagnosis to the third day corresponds to the expected function of NLR and ROS in the advancement of sepsis. NLR quantifies the ratio of neutrophils to lymphocytes in peripheral blood, linking the body's innate and adaptive immune systems. Any illness characterized by tissue injury activating systemic inflammatory response syndrome (SIRS) is associated with the increase in NLR. SIRS inhibits neutrophil apoptosis and enhances neutrophil-mediated death as part of the innate immune response. The elevated neutrophil count and reduced lymphocyte count in this scenario suggest the elevated NLR [11–13].

Sepsis pathophysiology involves PAMPs, endo- and exotoxins, lipids, and DNA. DAMPs, released by endogenous chemicals during early sepsis, activate TLRs on APCs and monocytes, translocating genes involved in inflammation, metabolism, and adaptive immune response to cause sepsis. Progressive damage and multi-organ malfunction arise from these mechanisms. NF κ B signal transduction occurs when PAMPs and DAMPs bind to TLRs on APCs and monocytes, leading to increased production of pro-inflammatory cytokines (IL) such IL-1, IL-12, IL-18, TNF- α , and interferon. Increased cytokine activation, complement pathways, coagulation, negative feedback, and impaired adaptive immune system regulation occur. Due to PAMP or DAMP activation, neutrophils have a lower oxidative burst capacity [14].

Studies have linked higher NLR to increased ROS in sepsis patients in the ICU/HCU. The table 2 indicates the moderate connection between NLR ($r = 0.442$; $p = 0.009$) and ROS on the first day of sepsis diagnosis in the ICU/HCU ($r = 0.400$ – 0.599). After three days of sepsis diagnosis, NLR ($r = 0.451$; $p = 0.007$) showed a significant correlation with ROS at the significance level of $p < 0.05$, with the moderate strength of correlation ($r = 0.400$ – 0.599). In the scatterplot graph (figure 1a), a linear trend line from the bottom left to the top right shows that increasing NLR examination results are connected with increased ROS examination results on the third day of sepsis diagnosis in the ICU/HCU.

Neutrophils mediate functional responses, including ROS generation, which may explain the positive association between NLR and ROS. ROS activates granular proteases and helps generate NETs. ROS generation leads to downstream actions such cytoplasmic granule release and pro-inflammatory cytokine synthesis, including TNF α and MIP-2. T. Veenith T. et al. found that ROS production is highly correlated with neutrophil cell count, suggesting that high ROS levels in blood samples actively indicate ICU patients with sepsis and may be useful for monitoring and treating sepsis [15].

The increase (delta) in NLR and ROS on the third day since the first day is also related to the pathophysiology of sepsis. Although inconsistent results have been reported by existing studies, NLR has been used in critical care treatment and can be a valuable prognostic tool for patients with sepsis. The early increase (< 6 hours) in NLR after acute physiological stress

may give NLR a role as an early marker of acute stress compared to other laboratory parameters (e.g., white blood cell count, bacteremia, CRP).

The research results demonstrate the correlation between the changes (delta) of both parameters. The table 4 demonstrates the positive and significant connection between the change in ROS and the delta change in NLR ($r = 0.496$; $p = 0.003$) at the significance level of $p < 0.05$. The correlation strength falls into the moderate range ($r = 0.400$ – 0.599). The scatterplot graph in figure 3a displays a linear trend line that runs from the bottom left to the top right, suggesting a tendency for rising delta NLR examination results to be correlated with rising delta ROS examination results. As a result, changes in ROS can be predicted based on increases in NLR.

Additionally, a linear trend line from the bottom left to the top right illustrates their correlation in the scatterplot graphs in figures 1a and 3a. This indicates a tendency for increasing NLR examination results associated with increasing ROS examination results on the first day of sepsis diagnosis in the ICU/HCU. Therefore, on the first and third days following the sepsis diagnosis in the ICU/HCU, the rise in NLR can predict the increase in ROS.

M. Laforge et al. used NLR to predict ROS levels in critically ill COVID-19 patients. Extra ROS from neutrophils increases the host's immunopathological response and disease development. Neutrophils release NETs, migrate quickly to target tissues, and generate and release ROS when danger signals are detected. Neutrophil dysregulation can transfer local inflammatory reactions to systemic processes through excessive ROS generation [16]. NLR predicts ROS and patient prognosis due to its correlation with disease severity and poor patient outcomes. In 2021, J. Song et al. found that NLR increased overall mortality (HR 1.14, 95% CI 1.10–1.17, per NLR quartile). Another study from Rotterdam found that NLR levels independently and strongly increased all-cause mortality (HR 1.64; 95% CI 1.44–1.86) [17].

Sepsis research showed similar results. Z. Huang et al. found that sepsis patients' NLR values were considerably greater in non-survivors and independent predictors of outcome. NLR appears to be a reliable sepsis prognostic predictor [18]. Another study by Spoto et al. found that the NLR value of 9.05 for sepsis diagnosis predicted 90-day mortality well. Combining it with MR proADM (PPV 52% and NPV 50%) and clinical sepsis scores like SIRS, qSOFA, and SOFA (PPV 96% and NPV 88%) improved it further [19].

Patients with septic shock may receive inotropic medications to boost cardiac contractility and vasopressors to improve vascular tone. VIS quantifies vasoactive inotropic medication use by providing equal weight to each drug based on potency [20, 21].

In the table 1, VIS examination results for sepsis-diagnosed patients in the ICU/HCU were 56.84 ± 118.77 on the first day (pretest) and 62.65 ± 126.07 on the third day (posttest). The average VIS increased by

10.2% (5.81 ± 105.50). The first day (pretest) ROS findings for sepsis-diagnosed patients in the ICU/HCU averaged 231.65 ± 96.46 , while the third day (posttest) averaged 237.88 ± 102.55 . The average rise in ROS was 6.24 ± 79.82 , or 2.7%. Both VIS and ROS levels increased from the first day (pretest) to the third day (posttest) following sepsis diagnosis, indicating disease progression.

Oxidative stress plays a crucial role in the pathogenesis of sepsis and is associated with decreased survival. However, reliable ROS-related biomarkers for predicting survival and assessing therapy response in sepsis are still lacking. The research by C. Bime et al. in an independent cohort validated the use of ROS to predict survival in patients [22].

Similarly to the increased levels of ROS correlating with disease progression and serving as one of the predictors of survival, the same findings are demonstrated by VIS. VIS shows that elevated ROS levels correlate with illness progression and survival. Vasoactive and inotropic medicines can cause cardiac arrhythmias, ischemia, and hypo/hypertension, despite their usage in patient care. In 2021, J. Song et al. found a link between high VIS and poor outcomes. A. Belletti et al. found that large dosages of vasoactive medications, which are indications of illness severity, increase mortality rates. Specifically, the need for vasoactive drugs is included in mortality prediction scores [17,23].

Therefore, there is consistency between the findings of increased ROS and high VIS in sepsis patients. VIS shows that elevated ROS levels correlate with illness progression and survival. Vasoactive and inotropic medicines can cause cardiac arrhythmias, ischemia, and hypo/hypertension, despite their usage in patient care. In 2021, J. Song et al. found a link between high VIS and poor outcomes. A. Belletti et al. found that large dosages of vasoactive medications, which are indications of illness severity, increase mortality rates. Mortality prediction scores incorporate vasoactive medication use.

Increased ROS and elevated VIS in sepsis patients are consistent. This explains why VIS correlates positively with ROS in study. At the initial sepsis diagnosis in the ICU/HCU, VIS ($r = 0.460$; $p = 0.006$) correlated positively and significantly with ROS ($p < 0.05$), with a moderate correlation strength ($r = 0.400$ – 0.599). In the posttest on day three, VIS ($r = 0.654$; $p = < 0.001$) correlated favourably and significantly with ROS ($r = 0.600$ – 0.799) at $p < 0.05$. The scatterplot graphs in the figure 1b and the figure 2b exhibit a linear trend from the bottom left to the top right, indicating that ROS examination results rise with VIS examination results.

The examination results indicated that the average rise in VIS, or delta, was 5.81 ± 105.50 , or 10.2%. Three trials, two of which involved pediatric populations, validated VIS in septic shock patients, according to the research by A. Belletti et al. In the largest trial, 138 children (aged 60 days to 18 years) who needed vasoactive support after being admitted to the intensive care unit

(ICU) due to sepsis were examined by McIntosh and colleagues. The study discovered that the best predictor of the main result was VIS at 48 hours [23].

The application of VIS in pediatric septic shock and general pediatric intensive care units (PICUs) has been supported by numerous studies. According to the study by D. Kallekkattu et al., death in pediatric septic shock could be independently predicted by IS > 28 and VIS > 42.5 , both of which exhibited good sensitivity and specificity [21]. A number of authors assessed the prognostic value of VISmax calculated in the emergency department (ED) in patients diagnosed with sepsis according to the Sepsis-3 definition. They found that although VISmax showed prognostic value comparable to SOFA and APACHE II scores, the sole use of VISmax had limited predictive value for 30-day mortality. In their study, the authors argued that high VIS could be a major contributor to poor outcomes [17].

Complement activation in progressive sepsis is linked to rising ROS levels, much as elevated VIS is observed in patients with severe sepsis. The scatterplot graphs in figures 2a and 5 of this study demonstrate a linear trend from the bottom left to the top right, suggesting that on the first and third days following the diagnosis of sepsis in the ICU/HCU, ROS examination findings increase in tandem with increases in VIS examination results. Therefore, on the third day following the diagnosis of sepsis in the ICU/HCU, elevated VIS may be used as a predictor of elevated ROS. The graphic data is supported by the results of data analysis in the table 4 where the delta change in VIS ($r = 0.583$; $p \leq 0.001$) is positively and significantly correlated with changes in ROS at the value of $p < 0.05$, where the strength of the correlation is in the medium category ($r = 0.400$ – 0.599). The scatterplot graph in the figure 3b shows a linear line from the bottom left to the top right, which means that there is a tendency for the delta of the VIS examination to increase, the more the delta results of the ROS examination for sepsis patients in the ICU/HCU increase, so it can be concluded that changes in VIS increase can be used as a predictor changes in ROS increase.

Patients diagnosed with sepsis in the ICU/HCU had the average CFB examination on the first day (pretest) of 353.00 ± 850.78 , and on the third day (posttest), the average was 436.91 ± 1210.58 , indicating the 23.8% rise in the average CFB. Patients diagnosed with sepsis in the ICU/HCU had the average ROS examination on day 1 (pretest) of 231.65 ± 96.46 , and on day 3 (posttest) of 237.88 ± 102.55 , indicating the delta rise of 6.24 ± 79.82 or 2.7%. This shows the increase in delta CFB and delta.

In the bivariate study in the table 2, CFB was a poor predictor of ROS on the first day of sepsis diagnosis in the ICU/HCU ($r = 0.000$ – 0.199). The scatterplot graph in the figure 1c displays a linear trend from the bottom left to the top right, showing that ROS examination findings rise with CFB values, but this is not statistically significant.

The reason for administering fluid bolus in septic shock is to restore circulating fluid volume and opti-

mize cardiac output. According to the Frank–Starling principle, the increase in preload leads to the increase in stroke volume, although myocardial dysfunction associated with sepsis can alter this relationship [24, 25]. Therefore, according to the theory, positive CFB on the first day may also contribute to sepsis improvement. This is supported by data from analyzing prospectively collected data from the Korean Sepsis Alliance Database, which registered 11,981 sepsis patients from 20 hospitals. It was found that positive CFB conditions on the first day had no significant difference in mortality on the 28th day (hazard ratio [HR], 1.17; 95% confidence interval [CI] 0.85–1.60; $P = 0.354$) between the two groups [26].

Meanwhile, several studies indicate a relationship between sepsis and increased levels of oxidative stress [22]. There is evidence that oxidative stress caused by reactive oxygen species (ROS) in sepsis is characterized by tissue ischemia-reperfusion injury and intense systemic inflammatory response. Based on this explanation, it is known that in the early management of sepsis, resuscitation aims to optimize fluid volume and cardiac function and improve the septic condition, thus not correlating with existing ROS levels [27–29].

This study also links CFB to ROS on day three. CFB ($r = 0.399$; $p = 0.019$) positive and significant linked with ROS at the p -value < 0.05 , with a modest association ($r = 0.200$ – 0.399). The figure 2c's scatterplot graph displays a linear line from the bottom left to the top right, demonstrating that CFB and ROS results increase on the third day following sepsis diagnosis in the ICU/HCU. The earlier research by Huang et al. (2019) demonstrated that CFB in the first 72 hours can predict septic shock survival [7]. Another study found that CFB after 72 hours after ICU admission (but not 24 hours) independently increases mortality risk [29, 30]. In the middle and late stages of sepsis, the pathogenesis and course are complex, and many factors, including patient physical conditions and complications, fluid types, and fluid resuscitation target endpoints can influence fluid management [7, 31, 32].

The mechanism by which positive CFB is associated with poor outcomes in septic patients remains unclear. However, the previously recommended conceptual model may help explain this. In the four stages of dynamic volume resuscitation (rescue, optimization, stabilization, and de-escalation), septic patients in a compensated state after initial rescue can enter the optimization stage to increase tissue perfusion and reduce organ dysfunction. Consequently, hypervolemia after the ICU rescue phase on the first day may exacerbate capillary leakage to organs, thereby contributing to organ dysfunction and subsequent organ failure [33].

According to this theory, in this study, the increase in delta change in CFB cannot be a good predictor for delta change in ROS because early fluid resuscitation and active and effective fluid volume management are crucial for the rescue and treatment of patients with sepsis. CFB during sepsis treatment increases mortality, especially after 24 hours. Overly vigorous early and

sustained fluid resuscitation or unmonitored maintenance fluid therapy might produce positive CFB. The release of pro-inflammatory mediators, ROS, and proteases by active neutrophils and excessive fluid extravasation leading to tissue and organ edema can increase vascular endothelial permeability and affect the patient's prognosis [32, 34].

Subsequently, multivariate analysis showed a significant relationship between delta NLR and delta VIS with delta ROS. The R-squared value of 0.420 suggests that NLR and VIS changes predict 42.0% of ROS changes. Delta NLR ($B = 2.060$) and delta VIS ($B = 0.298$) coefficients show that ROS increases by 2.060 and 0.298 units per unit of NLR and VIS, respectively. Both predictors achieve statistical significance ($p < 0.05$). NLR, VIS, and ROS levels are positively correlated, according to this study. The data do not support the theory that CFB increases ROS.

Our study has several clinical implications. Firstly, for clinical monitoring, using NLR and VIS as significant predictors of ROS changes provides valuable insight for clinicians in monitoring septic patients. Continuous assessment of these parameters can help identify individuals at risk of worsening oxidative stress. Secondly, the observed relationships provide a basis for exploring targeted interventions aimed at modulating NLR/VIS and optimizing hemodynamic support, potentially reducing oxidative stress in sepsis.

Nevertheless, it is crucial to recognize specific constraints in our research. Data collection was limited to the first and third days. The results indicate a rise in NLR, VIS, and ROS on the third day. However, due to

the limited timeframe, it was not possible to observe and analyze the biomarker changes over a longer period. In our study, we did not take into account other factors, such as the patient's clinical condition, that may have influenced the association between CFB and ROS. These factors include the patient's need for early fluid resuscitation and active management of fluid volume as part of their care. Furthermore, our study was constrained by a very small sample size and the fact that it was conducted at a single site. As a result, the applicability of our findings to broader patient populations is limited. The previous research has demonstrated that septic patients in the ICU/HCU exhibit varying features, thus emphasizing the need for caution in generalizing our results [35–37].

Subsequent investigations could prolong the study's timeframe and intensify the monitoring of variables to enhance the comprehensiveness and reliability of the data, as well as examine broader and more heterogeneous patient cohorts to enhance the generalizability of the conclusions.

Conclusions

This study demonstrates the significant correlation between NLR and VIS as predictive indicators of ROS on the third day following the first day. An increment of one unit in NLR results in the 2.060 unit increase in ROS, while a one unit rise in VIS leads to the 0.298 unit increase in ROS. There is a weak positive association between increased CFB and increasing ROS in sepsis patients, which deserves additional investigation.

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