Correlation between interleukin-6, HMGB-1, and neutrophil-to-lymphocyte ratio to acute respiratory distress syndrome

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Abstract

Background: **Acute respiratory distress syndrome (ARDS) has a high mortality rate if not diagnosed and treated promptly. Several markers could increase during ARDS, such as interleukin 6 (IL-6), high mobility group box-1 (HMGB-1), and neutrophil-to-lymphocyte ratio (NLR). This study aimed to determine the correlation between these markers and the severity of ARDS.**

***Methods*: This cross-sectional study was conducted at Dr. Wahidin Sudirohusodo General Hospital from January to April 2024. The population included in this study consisted of all patients treated in the Intensive Care Unit (ICU) and divided into two groups: ARDS and non-ARDS. IL-6, HMGB-1, and NLR were measured once during treatment. The severity assessment was conducted using the Berlin criteria (mild, moderate, severe) for ARDS patients.**

Results: **A total of 30 patients were divided equally into two groups. Mean IL-6 levels were higher in ARDS patients (21.15±3.89) than in non-ARDS (12.56±3.08) with a significant difference (p=0.000). The mean HMGB-1 levels were also higher in ARDS (26.46±30.14) than in non-ARDS (9.37±4.23), with a significant difference (p=0.040). IL-6 (p=0.000) and HMGB-1 (p=0.026) were also significantly associated with ARDS severity.**

***Conclusion*: IL-6 and HMGB-1 are related to ARDS severity.**

Key words: Acute respiratory distress syndrome, interleukin 6, high mobility group box-1, neutrophil-to-lymphocyte ratio.

Introduction

Acute respiratory distress syndrome (ARDS) is an acute lung disease that requires treatment in the Intensive Care Unit (ICU). ARDS has a high incidence in the ICU. The mortality rate for disease could reach up to 90%. However, early diagnosis and adequate treatment could reduce this number to 50%. (1)

Several cytokine markers and indicators might be found to be increased during ARDS. Interleukin 6 (IL-6) is a mediator often used as a marker of increased inflammation. ARDS causes activation of alveolar macrophages, which triggers the immune system. Damage to the lung parenchyma causes an increase in several pro-inflammatory cytokines, including IL-6. (2) In addition, high mobility group box-1 (HMGB-1) is known to be a mediator of acute lung damage through signals from pro-inflammation acceleration. In particular, serum HMGB-1 concentration during ARDS is an independent predictor of mortality in the ICU. (3) HMGB-1 level increases in blood circulation due to tissue damage, and it is necessary to examine this level to predict the prognosis of ARDS. (4) Neutrophil-to-lymphocyte ratio (NLR) is a marker

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of systemic inflammatory response. (5) High NLR was associated with poor outcomes. (6)
Early identification of inflammatory mediators in predicting ARDS severity (mild, moderate, and severe) might be useful in early intervention to reduce patient mortality rates. Therefore, we aimed to determine the correlation between IL-6, HMGB-1, and NLR with ARDS severity.

Materials and methods

This cross-sectional study was conducted at Dr. Wahidin Sudirohusodo General Hospital, Makassar, Indonesia, in January─April 2024. The population included in this study were all patients treated in the ICU of Dr. Wahidin Sudirohusodo General Hospital, Makassar, Indonesia.
The study sample was the population that met the inclusion criteria, and the patients or their families agreed to participate in this study. The inclusion criteria in this study were all patients treated in Dr. Wahidin Sudirohusodo General Hospital, Makassar, Indonesia. The exclusion criteria were patients with incomplete study data. The dropout criteria were patients who had a history of congenital lung disease, patients who withdrew during the study, and patients who passed away during the study period.
Patients or families of patients who were included as study subjects were given an oral or written explanation regarding the aims and procedures of the study and had signed an informed consent and agreed to participate in the study. Before the study was conducted, the researchers requested information on ethical clearance from the Ethics Committee for Biomedical Research on Humans, Faculty of Medicine, Hasanuddin University and Dr. Wahidin Sudirohusodo General Hospital, Makassar, Indonesia.
Patients included in the study were examined for HMGB-1, IL-6, and NLR and were assessed for their ARDS severity. The examination sample was blood from a vein using a 5-cc syringe and placed in serum-separating tubes (SST). The blood sample was allowed to clot for 30 minutes before being centrifuged at a speed of 3000 rpm for 15 minutes. The separated serum was placed in a microtube. Then, the sample was stored in a freezer at a temperature of <-20 °C and sent to the Hasanuddin University laboratory for examination. We assessed the severity of ARDS according to the Berlin criteria (mild: arterial oxygen pressure to inspired oxygen fraction ratio [PaO2/FiO2] <300, moderate: PaO2/FiO2 <200, and severe: PaO2/FiO2 <100).
The data obtained was collected using a prepared study data formula and arranged in a master table using the Microsoft Excel program. The study data was then processed using SPP version 25.0 software.
The normality test was conducted to determine the distribution of numerical data using the Kolmogorov-Smirnov test. Next, bivariate analysis was performed for variables with unpaired numerical data types in both groups. If the data was normally distributed, then the independent t-test was used. If the data was abnormally distributed, then the test used was the Mann-Whitney test. Statistical analysis of the correlation between ARDS severity and IL-6, HMGB-1, and NLR used the one-way ANOVA test if the data was normally distributed or the Kruskal-Wallis test as an alternative. A p-value<0.05 indicated significance.

Results

The total number of subjects in this study was 30. The study subjects were divided into two groups: ARDS and non-ARDS. In the group of ARDS patients, the majority were patients aged >45 years, with eight people (54%), and the majority were men, with eight people (54%) (**Table 1**).
The mean IL-6 level (**Table 2**) was higher in patients with ARDS (21.15±3.89) compared to non-ARDS (12.56±3.08). This difference was also statistically significant (p=0.000). The mean HMGB-1 level (**Table 3**) was higher in patients with ARDS (26.46±30.14) compared to non-ARDS patients (9.37±4.23). This difference was also statistically significant (p=0.040). The mean NLR was higher in the ARDS group (17.60±12.46) compared to the non-ARDS group (17.20±17.13) (**Table 4**). However, this difference was not significant (p=0.75).
Only IL-6 (p=0.000) and HMGB-1 (p=0.026) were significantly related to ARDS severity. These results indicated that the more severe the ARDS, the higher the IL-6 and HMGB-1 values will be (**Table 5**).

Discussion

IL-6 levels in the ARDS patient group were significantly higher than non-ARDS patients. These results were supported by a study that reported increases in IL-6, tumor necrosis factor α (TNF-α), and IL-1β in patients detected early with ARDS. (7) Activated macrophages release IL-6 and integrate signals made early in the inflammatory response since TNF-α and IL-1β partially activate them. (8) The increase of IL-6 and procalcitonin (PCT) was significantly higher in the ARDS group with multiple organ dysfunction (MOD). The sensitivity of PCT and IL-6 examination was very high, but the

specificity was low. Also, PCT and IL-6 assessments are almost related to the patient's life expectancy. The lower the levels of PCT and IL-6, the higher the life expectancy. PCT and IL-6 are a combination of sensitivity in MOD patients with complications of ARDS, which are biological markers that predict the incidence of ARDS. (9)

HMGB-1 level was also significantly higher in the ARDS patient group. Similar results in a study reported increased serum HMGB-1 and soluble urokinase plasminogen activator receptor (suPAR) in patients with ARDS, which were positively associated with inflammatory markers. (10) Another study in Japan examined HMGB-1 and HMGB-2 in 21 ARDS patients whose epithelial lining fluid (ELF) samples were taken during bronchoscopy examination. The results were strongly positive for HMGB-1 and HMGB-2 in all samples of ELF and plasma examinations. In contrast, control patients were found to be typically positive for ELF, although it was absent in plasma. (11)

HMGB-1 is a nuclear protein that also functions as an inflammatory cytokine. HMGB-1 is released in the latent phase of the inflammatory response, and the release of HMGB-1 from macrophages is induced by lipopolysaccharide (LPS), TNF-α, and IL-1 β stimulation. HMGB-1 contributes to acute lung injury activated by the nuclear factor kappa B (NF–κB) signaling pathway and induces inflammatory cytokines. A study with experimental animals found that lung injury induced by HMGB-1 affected the expression of forkhead box P3 (FOXP3) and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) via toll-like receptor 4 (TLR4), reducing regulatory T (Treg) cells' immunosuppressive function. (12)

The current study found a significant correlation between IL-6 level and ARDS severity. These results were supported by a study emphasizing the importance of examining cytokines such as IL-6 in patients with ARDS to predict clinical outcomes. In ARDS patients who had a high mortality rate, IL-6 levels were increased significantly, with a predicted mortality percentage of 94%. (13)

In this study, a significant correlation was also found between HMGB-1 levels and the severity of ARDS. These results were in accordance with a previous study, which reported an increase in HMGB-1 levels in pneumonia patients complicated by ARDS and was positively related to the severity of the disease. (14)

Conclusion

The more severe the inflammation, characterized by increasing levels of IL-6 and HMGB-1, the more severe ARDS. Hence, high IL-6 and HMGB-1 levels could be used to predict unfavorable outcomes in ARDS. Further studies are needed to assess IL-6, HMGB-1 levels, and NLR before and after ARDS. Cut-off values of IL-6 and HMGB-1 levels should be determined to predict ARDS outcome.

Conflict of interest

The authors certify no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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Table 1. Characteristics of the study subjects

Characteristic	Non-ARDS		ARDS	
	n	%	n	%
Age				
- <25 years	6	40%	3	20%
- ≥26-<45 years	3	20%	4	26%
- ≥45 years	6	40%	8	54%
Sex				
- Female	9	60%	7	46%
- Male	6	40%	8	54%

Legend: ARDS=acute respiratory distress syndrome.

Table 2. IL-6 levels in ARDS and non-ARDS patients

Group	n (%)	IL-6 level (pg/ml)	p-value
ARDS	15 (50%)	12.44±19.47	0.000*
Non-ARDS	15 (50%)	1.54±0.31	

Legend: IL-6=interleukin 6; ARDS=acute respiratory distress syndrome.
*significant.

Table 3. HMGB-1 levels in ARDS and non-ARDS patients

Group	n (%)	HMGB-1 level (pg/ml)	p-value
ARDS	15 (50%)	26.46±30.14	0.040*
Non-ARDS	15 (50%)	9.37±4.23	

Legend: HMGB-1=high mobility group box-1; ARDS=acute respiratory distress syndrome.
*significant.

Table 4. NLR in ARDS and non-ARDS patients

Group	n (%)	NLR	p-value
ARDS	15 (50%)	17.60±12.46	0.750**
Non-ARDS	15 (50%)	17.20±17.13	

Legend: NLR=neutrophil-to-lymphocyte ratio; ARDS=acute respiratory distress syndrome.
**not significant.

Table 5. Correlation between IL-6, HMGB-1 levels, and NLR with ARDS severity

ARDS severity	Non-ARDS	Mild	Moderate	Severe	p-value
n (%)	15 (50%)	5 (16.6%)	5 (16.6%)	5 (16.6%)	
IL-6 level (pg/ml)	1.54±0.31	1.92±0.49	2.33±0.12	33.09±22.99	0.000*
HMGB-1 level (pg/ml)	9.37±4.23	14.27±12.35	9.98±2.15	55.14±38.33	0.026*
NLR	17.20±17.13	16.80±8.07	24.20±18.88	11.80±4.91	0.940**

Legend: IL-6=interleukin 6; HMGB-1=high mobility group box-1; NLR=neutrophil-to-lymphocyte ratio; ARDS=acute respiratory distress syndrome
*significant; **not significant.

References

1. Sinatra J, Sianturi IL, Tambunan R, Siahaan DL, Siahaan JM. Faktor-faktor yang Mempengaruhi Kematian pada Pasien ARDS di ICU. Purbalingga, Indonesia: Eureka Media Aksara;2022.
2. Gandini L, Fior G, Schibler A, Obonyo NG, Bassi GL, Suen JY, et al. Interleukin-6 inhibitors in non-COVID-19 ARDS: analyzing the past to step into the post-COVID-19 era. Crit Care 2023;27:124.
3. Tasaka S. Acute Respiratory Distress Syndrome. Advances in Diagnostic Tools and Disease Management. Singapore: Springer Singapore; 2022 [cited 2023 Sep]. Available from: https://link.springer.com/book/10.1007/978-981-16-8371-8
4. Young MD, Cancio TS, Thorpe CR, Willis RP, Snook JK, Jordan BS, et al. Circulatory HMGB-1 is an early predictive and prognostic biomarker of ARDS and mortality in a swine model of polytrauma. Front Immunol 2023;14: 1227751.
5. Yang L, Gao C, He Y, Wang X, Yang L, Guo S, et al. The Neutrophil-to-Lymphocyte Rasio is Associated with the Requirement and the Duration of Invasive Mechanical Ventilation in Acute Respiratory Distress Syndrome Patients: A Retrospective Study. Can Respir J 2022; 2022:1581038.
6. Mehdi KB, Jehan S, Akhter M, Angez M, Nasir N. Utility of neutrophil to lymphocyte rasio to predict acute respiratory distress syndrome in COVID-19: short communication. Ann Med Surg (Lond) 2023;85:3232-6.
7. Meduri GU, Headley S, Kohler G, Stentz F, Tolley E, Umberger R, et al. Persistent Elevation of Inflammatory Cytokines Predicts a Poor Outcome in ARDS. Plasma IL-1 beta and IL-6 Levels are Consistent and Efficient Predictors of Outcome Over Time. Chest 1995;107:1062-73.
8. Yousef IA, Mohamed AH, Mohamed GF, Abbas NI. Interleukin 6 in Diagnosis of ARDS Phases. Eur Chem Bull [Internet]. 2023 [cited 2023 Sep];12:2604-6. Available from: https://naeeem.shop/issue-content/interleukin-6-in-diagnosis-of-ards-phases-11683
9. Hui L, Zhang X, An X, Li J, Zang K, Shang F, et al. Higher serum procalcitonin and IL-6 levels predict worse diagnosis for acute respiratory distress syndrome patients with multiple organ dysfunction. Int J Clin Exp Pathol 2017;10: 7401-7.
10. Fan Y, Ye Z, Tang Y. Serum HMGB-1 and soluble urokinase plasminogen activator receptor levels aid diagnosis and prognosis prediction of sepsis with acute respiratory distress syndrome. Biomark Med 2023;17:231-9.
11. Ueno H, Matsuda T, Hashimoto S, Amaya F, Kitamura Y, Tanaka M, et al. Contributions of high mobility group box protein in experimental and clinical acute lung injury. Am J Respir Crit Care Med 2004;170:1310-6.
12. Zhou M, Zhang Y, Tang R, Liu H, Du M, Gao Z, et al. HMGB-1/TLR4 Signaling Affeccts Regulatory T Cells in Acute Lung Injury. J Inflamm Res 2021;14:1551-61.
13. Swaroopa D, Bhaskar K, Mahathi T, Katkam S, Raju YS, Chandra N, et al. Association of serum interleukin-6, interleukin-8, and Acute Physiology and Chronic Health Evaluation II score with clinical outcome in patients with acute respiratory distress syndrome. Indian J Crit Care Med 2016;20:518-25.
14. Gao Y, Liu M, Zhang H. The Relationship Between the Serum High-Mobility Group Protein B1 (HMGB-1) Level with the Severity of the Patient's Condition in Different Severe Pneumonia Patients Complicated with Acute Respiratory Distress Syndome (ARDS). Int J Clin Exp Pathol 2016;9:11989-92.