Medica Hospitalia
Journal of Clinical Medicine
Med Hosp 2024; vol 11 (1) : 7–12

OPEN ACCESS

Original Article

Analysis of the Relationship between Neutrophils, Lymphocytes, and Comorbidities with Time to Death in COVID-19 Patients

Hendra Tanuwijaya, Susanthy Djajalaksana, Rezki Tantular

Department of Pulmonology and Respiratory Medicine, Faculty of Medicine, Brawijaya University/
Saiful Anwar Regional General Hospital, Malang, Indonesia

p-ISSN: 2301-4369 e-ISSN: 2685-7898
https://doi.org/10.36408/mhjcm.v11i1.932

Accepted: February 27th, 2023
Approved: December 19th, 2023

Author Affiliation:
Department of Pulmonology and Respiratory Medicine, Brawijaya University/ Saiful Anwar Regional General Hospital, Malang, Indonesia

Author Correspondence:
Hendra Tanuwijaya
Jaksa Agung Suprapto Street No.2, Klojen, Malang, East Java 65111, Indonesia

E-mail:
dr_hendratan@student.ub.ac.id

Abstract

Background : COVID-19 caused 5.4 million deaths in 2021. Various parameters such as comorbidities and laboratory findings are known to be predictors of death in COVID-19, but these findings differ in each country, and their relationship to time of death has not been widely studied. This study was conducted to determine the relationship between levels of neutrophils, lymphocytes, neutrophil to lymphocyte ratio (NLR), and comorbidities with time to death in COVID-19.
Methods : A retrospective cohort study was conducted from April 2020 to September 2021 in the COVID-19 Inpatient Room at RSUD dr. Saiful Anwar Malang with 300 COVID-19 patient subjects aged 18 years and over. Data on comorbidities (hypertension, CAD, HF, obesity, COPD, CKD, cancer), time to death, and laboratory were taken from medical records. The Kolmogorov-Smirnov test, Chi square test and Mann Whitney test were carried out to analyze the data.
Results : There was a significant relationship between neutrophil levels and time to death, as well as a significant difference in neutrophil levels in the group with time to death >48 hours with comorbidities compared to <48 hours with and without comorbidities. There was a significant difference in lymphocyte levels and NLR in patients with comorbidities with time to death <48 hours and >48 hours ($p < 0.05$).
Conclusion : There is a significant relationship between neutrophil levels and time to death, both in subjects with and without comorbidities showing the potential of neutrophil levels as a predictor of time to death in COVID-19.

Keywords : COVID-19, time to death, comorbidities, neutrophils, lymphocytes

INTRODUCTION

COVID-19 caused around 5.4 million deaths worldwide in 2021. Although most COVID-19 sufferers experience mild flu-like symptoms, others can experience respiratory distress which can lead to a serious condition.[1] Various factors can be predictors of COVID-19 mortality such as comorbidities and laboratory parameters. However, studies related to predictors of COVID-19 mortality show different findings in various countries.[2]

Neutrophils and lymphocytes are laboratory parameters that have been proven to be associated with the severity of COVID-19. This laboratory parameter is also represented by the neutrophil to lymphocyte ratio (NLR). Neutrophils specifically have also been linked to the severity of COVID-19 via increased inflammation and thrombosis due to the production of neutrophil extracellular traps (NET). Studies show a significant relationship between NLR and the severity of COVID-19,[3] as well as an increased risk of mortality with increasing NLR.[4] Apart from NLR, previous studies have shown a significant association between lymphocytes and an increased risk of death in COVID-19 patients.[5] These findings demonstrate the importance of the association of neutrophils and lymphocytes with mortality in COVID-19.

Various studies on predictors of COVID-19 mortality have been conducted in various countries.[6–8] However, it is necessary to examine more deeply in this mortality case, which factors are predictors of time of death. In this retrospective cohort study, the relationship between time of death and comorbidities, as well as laboratory parameters such as neutrophils, lymphocytes and neutrophil-to-lymphocyte ratio (NLR), were studied.

METHODS

This retrospective study was conducted in the COVID-19 Inpatient Room (incovit) RSUD Dr Saiful Anwar Malang from April 2020 to September 2021. The data selected is data from subjects diagnosed with COVID-19, complete medical record data, and the final outcome of treatment (survived or non-survived). Data analysis carried out was time to death after hospital admission (<48 hours and >48 hours), comorbidity, and laboratory. Conditions categorized as COVID-19 comorbidities according to CDC recommendations are heart disease (hypertension, CAD, and HF), obesity (BMI > 30 kg/m^2), COPD, CKD, and cancer.[9] The laboratory data analyzed were neutrophils and lymphocytes with number classification based on a reference tool (XE-2100, Sysmex, Germany).[10] Neutrophil to lymphocyte ratio (NLR) was obtained by dividing the percentage of neutrophils by lymphocytes. Normality analysis of data distribution was carried out using the Kolmogorov-Smirnov test. Categorical data were analyzed using the Chi Square test, numerical data were analyzed using the Mann Whitney test. Statistical analysis used the Statistical Package for the Social Sciences (SPSS).

RESULTS

In this study, there were 300 patient data divided into time to death (<48 hours & >48 hours) and comorbidities (yes & no). There were 124 patients with time to death <48 hours, and 176 patients with time to death >48 hours after admission. 112 patients had no comorbidities and 188 patients had comorbidities. Data on the distribution of subjects based on time to death and comorbidities are shown in Table 1.

Analysis of the percentage of neutrophils, lymphocytes and NLR was carried out in this study. The mean percentage of neutrophils in all subjects was 81.90 ± 8.22. The mean percentage of lymphocytes in all subjects was 11.55 ± 6.57. Meanwhile, the average NLR is 10.58 ± 8.71. There was no significant difference in neutrophils, lymphocytes, and NLR related to the presence or absence of comorbidities. However, there was significant difference in the percentage of neutrophils in the subject groups with different times of death shown in Table 2.

Classification of neutrophil and lymphocyte levels did not show any subjects with neutropenia or lymphocytosis in this study. There were 50 subjects with normal neutrophil percentages and 250 subjects with neutrophilia. Regarding lymphocyte classification, there were 75 subjects with normal lymphocyte percentages and 225 subjects with lymphopenia. There were significant differences in neutrophil classification in subjects with different times of death. Data can be seen in Table 3.

Data analysis then continued with differentiating groups between time of death and comorbidity. There were 21 patients with time to death <48 hours without comorbidities, 103 patients with time to death <48 hours with comorbidities, 91 patients with time to death >48 hours without comorbidities, and 85 patients with time to death >48 hours with comorbidities. Neutrophil levels in patients with time to death >48 hours with comorbidities were significantly different compared to patients with time to death <48 hours with and without comorbidities ($p < 0.05$). Lymphocyte levels and NLR in patients with time to death >48 hours with comorbidities were significantly different compared to patients with time to death <48 hours with comorbidities ($p < 0.05$) (Table 4).

There was a pattern of decreasing mean neutrophils in patients with time to death <48 hours without and with comorbidities (83.61 vs 83.13) and continued in patients with time to death >48 hours without and with comorbidities (81.98 vs 79.91). In lymphocytes, there was a pattern of a slight decrease in

TABLE 1
Subject division based on time of death and comorbidities

Parameter		Time of Death		*p**
		<48 hours (N=124)	>48 hours (N=176)	
Comorbidities	Yes	103	85	0.001*
	No	21	91	

*Statistically significant differences (p<0.05)

TABLE 2
Neutrophil, lymphocyte and NLR levels based on time of death

Parameter (Mean±SD)	Time of Death		*p**
	<48 hours (N=124)	>48 hours (N=176)	
Neutrofils (%)	83.21 ± 7.41	80.98 ± 8.64	0.044*
Lymphocytes (%)	10.50 ± 5.78	12.29 ± 7.01	0.056
NLR	11.88 ± 10.72	9.67 ± 6.85	0.050

*Statistically significant differences (p<0.05)

TABLE 3
Classification of neutrophils and lymphocytes based on time of death

Parameter		Time of Death		*p**
		<48 hours (N=124)	>48 hours (N=176)	
Neutrofils (%)	Normal	13	37	0.016*
	Neutrophilia	111	139	
Lymphocytes (%)	Normal	27	48	0.279
	Lymphopenia	97	128	

*Statistically significant differences (p<0.05)

mean lymphocytes in patients with time to death <48 hours without and with comorbidities (10.63 vs 10.47) and continued with an increase in patients with time to death >48 hours without and with comorbidities (11.86 vs 12.77). A pattern of decreasing mean NLR was found in patients with time to death <48 hours without and with comorbidities (14.18 vs 12.68) and in patients with time to death >48 hours without and with comorbidities (10.03 vs 9.27).

DISCUSSION

In this study, a significant relationship was found between the presence or absence of comorbidities and time to death (p <0.05). Various studies also show similar findings related to comorbidities and mortality rates in COVID-19 patients. A study by Djaharuddin *et al*. shows that comorbidities such as diabetes, heart disease and hypertension are the most frequently found comorbidities in COVID-19 death cases, with more than 50 percent of sufferers of these death cases having two or more comorbidities.[11] A meta-analysis study in 2021 also showed similar results, with hypertension, diabetes, chronic obstructive pulmonary disease (COPD), and heart disease as the comorbidities most frequently associated with mortality in COVID-19 patients. The presence or absence of comorbidities is an important factor related to COVID-19 mortality due to the occurring pathological process. Metabolic syndrome comorbidities such as hypertension and diabetes are conditions that can

TABLE 4
Neutrophil and lymphocyte levels based on time of death and comorbidities

	Comorbidities	Time of Death		*p**
		<48 hours (N=124)	>48 hours (N=176)	
Neutrophil Percentage (Mean ± SD)	Yes	83.13 ± 7.05	79.91 ± 8.57[ab]	0.032*
	No	83.61 ± 9.16	81.98 ± 8.64	
Lymphocytes Percentage (Mean ± SD)	Yes	10.47 ± 5.23	12.77 ± 6.89[b]	0.111
	No	10.63 ± 8.10	11.86 ± 7.12	
NLR (Mean ± SD)	Yes	11.42 ± 10.28	9.27 ± 7.27[b]	0.089
	No	14.18 ± 12.68	10.03 ± 6.45	

[a](p<0.05 vs <48 hours without comorbidities); [b](p<0.05 vs <48 hours with comorbidities)

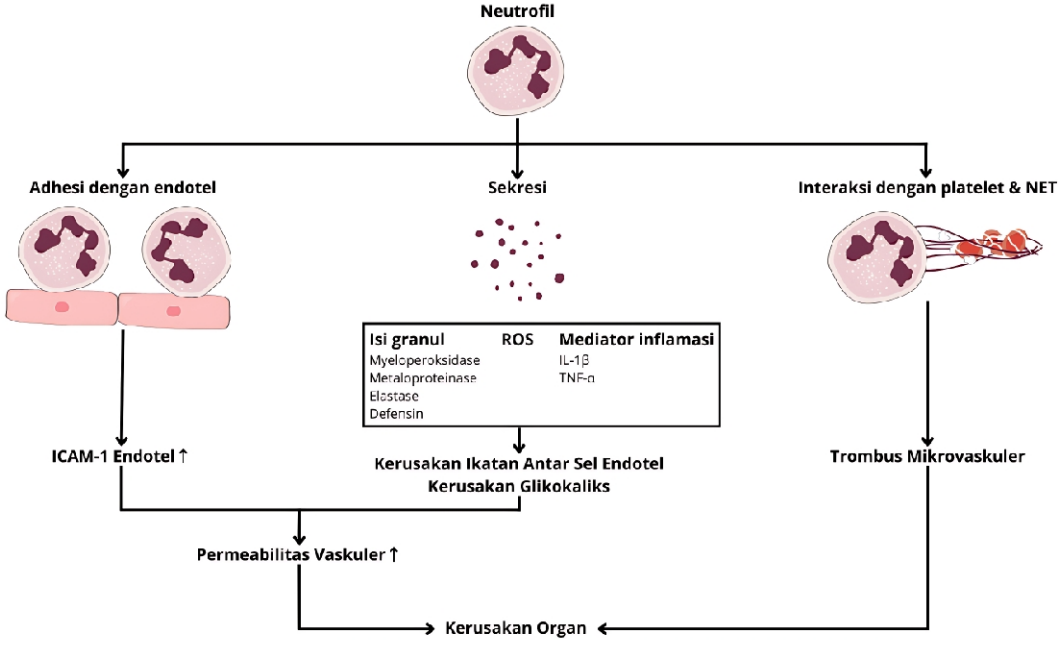

Figure 1. Pathophysiology of neutrophils in causing organ damage which leads to mortality. Neutrophil adhesion to the endothelium increases ICAM-1 which leads to increased transendothelial permeability, while neutrophil secretion results in increased paraendothelial permeability through damage to the glycocalyx and inter-endothelial bonds. The interaction of neutrophils with platelets and NET secretion causes microvascular thrombi which can lead to vascular obstruction. ICAM1: intercellular adhesion molecule-1; ROS: reactive oxygen species; IL: interleukin; TNF: tumor necrosis factor; NET: neutrophil extracellular traps. Licensed by Hendra Tanuwijaya

cause pathological conditions in various aspects, such as chronic inflammatory conditions, as well as vascular disorders that can contribute to COVID-19 mortality. Heart disease and COPD can also contribute to the process of dyspnea which is one of the main symptoms of COVID-19 which also contributes to the mortality of COVID-19 patients.[12]

In this study, significant differences in neutrophil levels were found in groups with different times of death (p<0.05). Apart from that, there were also significant differences in the categories of neutrophil levels (normal and neutrophilia) in groups with different times of death (p<0.05). Further analysis by dividing the subject groups based on time of death as well as comorbidities also showed significant differences in neutrophil levels (p<0.05). A previous study by Siahaan *et al*. shows similar results. In this study, neutrophil levels were significantly related to the clinical outcomes of COVID-19 patients.[13]

Previous studies also indirectly showed similar findings, where NLR was known to be significantly related to, as well as being a predictor and independent risk factor for mortality in COVID-19 patients.[4,14,15]

The findings regarding neutrophil levels in this study may also be related to its specific pathophysiology in COVID-19. Increased neutrophils have been proven to be one of the pathophysiology of COVID-19 with severe symptoms.[16] The function of neutrophils is to produce NETs which have also been shown to increase in COVID-19 patients.[17] Studies have proven that NETs have not only been shown to increase in the plasma of COVID-19 patients, but are also associated with severity, and have been found to increase in the lung tissue of COVID-19 patients.

NETs are bonds consisting of chromatin, pathogenicidal proteins, and oxidant enzymes secreted by neutrophils to fight infection. However, studies prove that NETs can have adverse effects such as increased inflammation and thrombosis if not regulated properly. In inflamed tissue, neutrophils and platelets can induce immunothrombosis, forming a fibrin filter that functions to capture pathogens.[18] NETs secreted by neutrophils into the intravascular can also cause microvascular obstruction without fibrin which can lead to the death of vascularized tissue cells.[19]

Immune defense by neutrophils can cause a variety of other damages. Metalloproteases, myeloperoxidase, and ROS secreted by neutrophils are also known to damage the glycocalyx that protects endothelial cells from exposure to intravascular circulating cells. [20] Damage to the glycocalyx facilitates neutrophil adhesion to the endothelium, leading to an increase in intercellular adhesion molecule 1 (ICAM-1). Increased ICAM-1 increases transendothelial permeability through caveolae. [21] Excessive neutrophil secretory activity such as secretion of neutrophil granule contents (neutrophil elastase and defensin), ROS, NET, and inflammatory mediators such as TNF-α are known to cause increased permeability of the endothelium through damage to the glycocalyx and intercellular bonds. [22] Together with increased microvascular thrombus formation, increased vascular permeability in COVID-19 due to increased neutrophil number and activity leads to organ damage and mortality (Figure 1). This explains the association of high neutrophil levels which was found to be significantly associated with time to death in this study.

In contrast to neutrophils, findings regarding lymphocytes in this study did not show significant differences in lymphocyte levels and time to death, although lymphocyte levels in patients with time to death <48 hours were lower than >48 hours. Categorical analysis dividing subject data into lymphopenia and normal also showed no significant differences. However, lymphocyte levels were found to be significantly higher in patients with comorbidities and time to death >48 hours compared to time to death <48 hours. These findings are in accordance with the study of Illg *et al.*, which analyzed lymphocytes in 311 COVID-19 patients. In this study, no significant relationship was found between lymphocytes and various variables such as age, intubation, and outcomes in the form of death. A total of 210 subjects had lymphocytes below normal, and 101 subjects had lymphocytes above normal.[23]

Previous studies by Zhang *et al.*, showed a pattern of lymphocyte decline that was consistent with the severity of COVID-19 disease. In the study with 2923 COVID-19 subjects, 70.2% showed low lymphocyte levels. In severe cases, 64.1% showed low lymphocytes; 85.0% in critical cases; and 93.5% of cases died. A pattern of gradual increase in lymphocytes was found in severe and critical cases, whereas lymphocyte levels in deceased subjects were consistently low.[24] Wang *et al.*'s study analyzed lymphocyte counts in 134 COVID-19 patients with severe symptoms. This study found a non-linear relationship between lymphocyte levels and the risk of death. COVID-19 patients with lymphocyte counts <0.95×109/L showed a significantly increased risk of death (seven-fold) compared to patients with lymphocyte counts >0.95×109/L. This shows the potential of lymphocytes as a predictor of death in COVID-19.[5]

The findings of NLR levels in this study are different from studies that have been conducted previously. In this study, NLR was not significantly associated with time to death in general, but was significantly associated with time to death in patients with comorbidities. The study of Toori *et al.* showed a significant association of NLR with COVID-19 disease severity.[3] Liu *et al.*'s study showed an increase in mortality risk of 8% for every 1 unit increase in NLR.[4] These studies demonstrate the potential of NLR as an easy and inexpensive predictor, as well as a risk factor, of mortality in COVID-19. The relationship between NLR and time to death in this study was almost significant ($p = 0.05$), indicating a possible association of the findings with study weaknesses such as small sample size and non-multicenter study. However, the finding of a significant relationship between NLR and time to death in patients with comorbidities shows the importance of patient comorbidities in changing the composition of leukocyte differentiation in COVID-19 patients.

In this study, significant differences in lymphocyte levels and NLR based on time of death were only found in patients with comorbidities. Various COVID-19 comorbidities stated by the CDC are known to be associated with a decrease in lymphocytes and an increase in neutrophils. The systemic stress response is known to increase catecholamines and cortisol which leads to changes in leukocyte differentiation. Leukocyte differentiation in this condition leads to a lower percentage of lymphocytes. This can occur due to

regulation of lymphocyte proliferation, increased lymphocyte apoptosis, and changes in lymphocyte distribution.[25]

This study has several weaknesses. First, the study only included one health facility center as a data source, namely Dr. Saiful Anwar Malang. Second, the number of research samples was only 300 patients. Third, time of death data was collected which was only based on the time of hospital admission, so it could not represent the patient's condition before entering the hospital, and the time of death was only divided by a time limit of 48 hours. It is hoped that more time-to-death divisions will provide a more detailed analysis of the time-to-death with lymphocytes and neutrophils of COVID-19 patients.

CONCLUSION

Based on this study, it can be concluded that there is a significant relationship between neutrophil levels and time to death in COVID-19 patients, both with and without comorbidities. In addition, a significant relationship was found between lymphocyte levels and NLR and time to death in COVID-19 patients with comorbidities.

REFERENCES

1. Lascarrou JB. COVID-19-related ARDS: one disease, two trajectories, and several unanswered questions. The Lancet Respiratory Medicine. 2021 Dec 1;9(12):1345–7.
2. Soto A, Quiñones-Laveriano DM, Azañero J, Chumpitaz R, Claros J, Salazar L, *et al*. Mortality and associated risk factors in patients hospitalized due to COVID-19 in a Peruvian reference hospital. PLOS ONE. 2022 Mar 2;17(3):e0264789.
3. Toori KU, Qureshi MA, Chaudhry A, Safdar MF. Neutrophil to lymphocyte ratio (NLR) in COVID-19: A cheap prognostic marker in a resource constraint setting. Pak J Med Sci. 2021;37(5):1435–9.
4. Liu Y, Du X, Chen J, Jin Y, Peng L, Wang HHX, *et al*. Neutrophil-to-lymphocyte ratio as an independent risk factor for mortality in hospitalized patients with COVID-19. J Infect. 2020 Jul;81(1):e6–12.
5. Wang S, Sheng Y, Tu J, Zhang L. Association between peripheral lymphocyte count and the mortality risk of COVID-19 inpatients. BMC Pulm Med. 2021 Feb 11;21:55.
6. Ottenhoff MC, Ramos LA, Potters W, Janssen MLF, Hubers D, Hu S, *et al*. Predicting mortality of individual patients with COVID-19: a multicentre Dutch cohort. BMJ Open. 2021 Jul 1;11(7):e047347.
7. Gopalan N, Senthil S, Prabakar NL, Senguttuvan T, Bhaskar A, Jagannathan M, *et al*. Predictors of mortality among hospitalized COVID-19 patients and risk score formulation for prioritizing tertiary care–An experience from South India. PLOS ONE. 2022 Feb 3;17(2):e0263471.
8. Shi C, Wang L, Ye J, Gu Z, Wang S, Xia J, *et al*. Predictors of mortality in patients with coronavirus disease 2019: a systematic review and meta-analysis. BMC Infectious Diseases. 2021 Jul 8;21(1):663.
9. CDC. People with Certain Medical Conditions [Internet]. Centers for Disease Control and Prevention. 2022 [cited 2022 Jul 9]. Available from: https://www.cdc.gov/coronavirus/2019-ncov/need-extra-precautions/people-with-medical-conditions.html
10. Sysmex. XE-2100 Automated Hematology System [Internet]. 2010. Available from: https://www.sysmex.com/la/pt/Products/Documents/English/XE-2100-English.pdf
11. Djaharuddin I, Munawwarah S, Nurulita A, Ilyas M, Tabri NA, Lihawa N. Comorbidities and mortality in COVID-19 patients. Gac Sanit. 2021;35:S530–2.
12. Dessie ZG, Zewotir T. Mortality-related risk factors of COVID-19: a systematic review and meta-analysis of 42 studies and 423,117 patients. BMC Infectious Diseases. 2021 Aug 21;21(1):855.
13. Siahaan MA, Purba SD, Amila A. Levels of Lymphocytes, Neutrophils, Leukocytes and Outcome of Covid-19 Patients. International Journal of Nursing and Health Services (IJNHS). 2022 Dec 27;5(6):511–8.
14. Fuad M, Oehadian A, Prihatni D, Marthoenis M. Neutrophil-to-Lymphocyte Ratio and Covid-19 Symptom-based Severity at Admission. Althea Medical Journal. 2021 Mar 31;8(1):1–6.
15. Li X, Liu C, Mao Z, Xiao M, Wang L, Qi S, *et al*. Predictive values of neutrophil-to-lymphocyte ratio on disease severity and mortality in COVID-19 patients: a systematic review and meta-analysis. Crit Care. 2020 Nov 16;24(1):647.
16. Chua RL, Lukassen S, Trump S, Hennig BP, Wendisch D, Pott F, *et al*. COVID-19 severity correlates with airway epithelium-immune cell interactions identified by single-cell analysis. Nat Biotechnol. 2020 Aug;38(8):970–9.
17. Middleton EA, He XY, Denorme F, Campbell RA, Ng D, Salvatore SP, *et al*. Neutrophil extracellular traps contribute to immunothrombosis in COVID-19 acute respiratory distress syndrome. Blood. 2020 Sep 3;136(10):1169–79.
18. Gaertner F, Massberg S. Blood coagulation in immunothrombosis-At the frontline of intravascular immunity. Semin Immunol. 2016 Dec;28(6):561–9.
19. Jiménez-Alcázar M, Rangaswamy C, Panda R, Bitterling J, Simsek YJ, Long AT, *et al*. Host DNases prevent vascular occlusion by neutrophil extracellular traps. Science. 2017 Dec 1;358(6367):1202–6.
20. Ali MM, Mahmoud AM, Le Master E, Levitan I, Phillips SA. Role of matrix metalloproteinases and histone deacetylase in oxidative stress-induced degradation of the endothelial glycocalyx. Am J Physiol Heart Circ Physiol. 2019 Mar 1;316(3):H647–63.
21. Hu G, Vogel SM, Schwartz DE, Malik AB, Minshall RD. Intercellular Adhesion Molecule-1Dependent Neutrophil Adhesion to Endothelial Cells Induces Caveolae-Mediated Pulmonary Vascular Hyperpermeability. Circ Res. 2008 Jun 20;102(12):e120–31.
22. Meegan JE, Yang X, Coleman DC, Jannaway M, Yuan SY. Neutrophil-Mediated Vascular Barrier Injury: Role of Neutrophil Extracellular Traps. Microcirculation. 2017 Apr;24(3):10.1111/micc.12352.
23. Illg Z, Muller G, Mueller M, Nippert J, Allen B. Analysis of absolute lymphocyte count in patients with COVID-19. Am J Emerg Med. 2021 Aug;46:16–9.
24. Zhang HJ, Qi GQ, Gu X, Zhang XY, Fang YF, Jiang H, *et al*. Lymphocyte blood levels that remain low can predict the death of patients with COVID-19. Medicine (Baltimore). 2021 Jul 16;100(28):e26503.
25. Nunez J, Minana G, Bodi V, Nunez E, Sanchis J, Husser O, *et al*. Low Lymphocyte Count and Cardiovascular Diseases. CMC. 2011 Jul 1;18(21):3226–33.