European Review for Medical and Pharmacological Sciences 2022; 26: 5963-5970

Are neutrophil-to-lymphocyte ratios and large unstained cells different in hospitalized COVID-19 PCR-positive patients with and without diabetes mellitus?

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Abstract. – OBJECTIVE: SARS-CoV-2 might present with multisystem involvement due to its entry into many cells with ACE2 receptors on their surfaces, such as heart, endothelial, and lung alveoli cells. Studies have indicated that COVID-19 infection causes a severe clinical presentation in diabetic patients due to dysregulation of the metabolic and immune systems. The hematological effects of COVID-19 and the relationship of lymphopenia with the severity of the disease have been reported previously. The parameter of percentage of large unstained cells (LUCs) reflects active lymphocytes and peroxidase-negative cells. The neutrophil-to-lymphocyte ratio (NLR) is another reliable marker of inflammation in cases of cardiac diseases, solid tumors, and sepsis. The present study aimed to evaluate whether the parameters of LUCs and NLR differed between diabetic and nondiabetic individuals with COVID-19. Associations with disease severity were also sought.

MATERIALS AND METHODS: In our retrospective study, the data of 1,053 patients [230 diabetic patients (21.83%) and 823 nondiabetic patients (78.15%)] were reviewed. The white blood cell (WBC) count, neutrophil count, neutrophil%, lymphocyte count, lymphocyte%, LUC count, %LUCs, NLR, platelet count, hemoglobin level, HbA1c, history of diabetes, surveillance during hospitalization, and pulmonary infiltration status within the first 24 hours after admission to the hospital were analyzed from the records.

RESULTS: When diabetic patients were compared with nondiabetics, the age [65 (20-90) *vs.* 42 (18-94) years], WBC count [6.72 (2.6-24.04) *vs.* 5.91 (1.35-52.68)], neutrophil count [4.29 (1.28-65) *vs.* 3.68 (0.02-50.47)], neutrophil% [67.53±12.3 *vs.* 64.08±13.28], NLR [3.35 (0.83-38.11) *vs.* 2.48 (0.01-68.58)], and LUC count [0.11 (0.03-0.98) *vs.* 0.1 (0.02-3.06)] of the diabetic group were found to be higher and these differences were statistically significant ($p<0.001$, $p<0.001$, $p<0.001$, $p<0.001$, $p<0.001$, and $p=0.015$, respectively).

CONCLUSIONS: We determined that LUC counts and NLR values in COVID-19-positive patients with diabetes were statistically significantly higher compared to nondiabetic patients.

Key Words:
COVID-19, Diabetes mellitus, Neutrophil-to-lymphocyte ratio, Large unstained cells.

Introduction

Coronavirus disease 2019 (COVID-19) has become the fastest-spreading disease worldwide, with over 519 million cases and 6 million deaths at the time of writing[1]. COVID-19 cases have been clinically divided into asymptomatic, mild, moderate, severe, and critical cases. It is a multisystemic disease as the development and severity of respiratory symptoms depend on factors including viral load, genetic factors, immune reactions, cytokine storms, and comorbidities[2].

Diabetes mellitus (DM) has been associated with mortality, acute respiratory distress syndrome (ARDS), and disease progression in patients with COVID-19[3-5]. The mechanisms underlying se-

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vere diabetic COVID-19 cases are impaired lung function, decreased neutrophil phagocytosis, a low-grade pro-inflammatory state in the body with increased secretion of cytokines, including interleukin (IL)-1, IL-6, IL-8, and tumor necrosis factor-α (TNF-α), T-cell imbalance (T-helper 17 increases with regulatory T-cell decreases), dysregulation of AMPK/mTOR signaling, and increased furin level[6]. SARS-CoV-2 was shown to negatively affect pancreatic beta cells. COVID-19 infection shifts the cellular metabolism to glycolysis, glutathione depletion causes oxidative damage, and the increase of interferon regulatory factor-5 causes activation of pro-inflammatory pathways in diabetic patients. Metabolic imbalance, deterioration of the immune system, and a tendency to a prothrombotic state can lead to poor prognosis in cases of COVID-19 infection in diabetic patients[7-9].

An autopsy series of COVID-19 patients revealed significantly shrunken spleens and decreased lymphocytes, macrophage proliferation and phagocytosis, depletion of lymphocytes in the lymph nodes, and a decrease in all hematopoietic cell lines in the bone marrow[10]. Five types of leukocytes are typically found in peripheral blood: neutrophils, lymphocytes, monocytes, eosinophils, and basophils. A routine hematology analyzer can be used to measure the percentage of large unstained cells (%LUCs) in addition to the white blood cell (WBC) population, reflecting activated lymphocytes and peroxidase-negative cells. The main problem with these cells to date has been their lack of specificity. LUCs may include blasts, atypical lymphocytes, plasma cells, and peroxidase-negative neutrophils. The %LUCs value has been found to increase significantly among untreated, asymptomatic, HIV-infected patients and it can be used as a marker of disease progression and immune activation[11,12]. There are also studies[13,14] reporting that the neutrophil-to-lymphocyte ratio (NLR) is a reliable marker for showing the severity of COVID-19 disease. The present study aimed to analyze whether there was a difference in LUC counts and NLR values in diabetic and nondiabetic COVID-19 PCR-positive patients and to investigate whether the LUC value might be a parameter indicating disease severity.

Materials and Methods

The data of 1,053 patients hospitalized in Ankara City Hospital's infectious diseases service and intensive care unit between March 15, 2020, and July 15, 2020, were reviewed retrospectively. The WBC count, neutrophil (NEU) count, neutrophil%, lymphocyte (LYM) count, lymphocyte%, LUC count, %LUCs, NLR, platelet (PLT) count, hemoglobin (Hb) value, hemoglobin A1c (HbA1c), history of diabetes, surveillance during hospitalization, and pulmonary infiltration status within the first 24 hours after admission to the hospital were analyzed from the records. COVID-19 PCR-positive patients over 18 years of age were included. Patients with hematologic and solid malignancies, systemic lupus erythematosus, splenectomy, chronic liver disease, chronic kidney disease, myelodysplastic syndrome, immune thrombocytopenic purpura, thalassemia, polycythemia vera, pregnancy, HIV infection, hepatitis B infection, rheumatoid arthritis, previous tuberculosis, a history of aspergillosis, and usage of immunosuppressant drugs were excluded.

Statistical Analysis

Continuous variables were expressed as mean±standard deviation and/or median (min-max) values, while categorical data were expressed as numbers and percentages. The Kolmogorov-Smirnov goodness-of-fit test was applied in the normality analysis of continuous variables and *t*-tests were used to analyze differences between independent groups for two groups with normal distribution. One-way analysis of variance (post-hoc: Bonferroni) was applied for analyses among three groups. The Mann-Whitney U test was used to analyze variables that did not comply with normal distribution between two groups, and the Kruskal-Wallis test (post hoc: Mann-Whitney U test with Bonferroni correction) was used for analysis among three groups. Comparisons of categorical data were performed with the chi-square test and Fisher exact test. Independent risk predictors based on possible factors identified in previous analyses were first examined using univariate logistic regression analysis (enter method). Age, HbA1c, WBC count, NEU count, NEU%, LYM, LYM%, NLR, and Hb values were evaluated in the context of significantly increasing the risk of admission to the intensive care unit. For the multivariate model, the NEU%, LYM%, NLR, and WBC variables were excluded since they were highly correlated with each other (r>0.70, VIF>5), and age, gender, HbA1c, NEU, LYM, and Hb, which were found to be statistically significant in univariate analyses or clinically significant, were analyzed by multiple logistic regression analysis (backward logistic regression). The Hosmer and Lemeshow test, omnibus tests of model coeffi-

Table I. Sociodemographic and clinical characteristics of the patients.

		n	%
Gender	Female	473	44.9
	Male	580	55.1
DM status	Non-DM	823	78.15
	Type 1 DM	2	0.18
	Type 2 DM	228	21.65
Hospitalization status	Ward	932	88.5
	Intensive care unit	67	6.4
	Deteriorating patients	54	5.1
Outcome	Recovering patients	1,016	96.5
	Death	37	3.5
Total		1,053	100.0

cients, and Nagelkerke R-square values were determined for model fit and statistical significance. All analyses were performed with IBM SPSS Statistics 26.0 (IBM Corp., Armonk, NY, USA), and the statistical significance level was accepted as $p<0.05$.

Results

The mean age of the 1,053 participating patients was 48.79±17.92 (min=18, max=94) years, the male/female ratio was 55.1% to 44.9, and 21.83% of the patients had diabetes mellitus (Type 1 DM: 0.18%, Type 2 DM: 21.65%). While 88.5% (n=932) of the patients were hospitalized in the ward, 6.4% (n=67) were directly treated in the intensive care unit and 5.1% (n=54) were transferred to the intensive care unit from the ward due to the worsening of their clinical conditions (Table I). While 96.5% of the patients were discharged upon recovery, 3.5% died during hospitalization. The mean NLR, LUC count, and percentages of the patients with and without diabetes are shown in Table II. The mean HbA1c value measured in 63.9% of the diabetic patients was 7.86±1.59 (min=5.6, max=13.2). Finally, 72% of the patients had infiltrative involvement findings on chest tomography.

The mean age of the patients was higher in the diabetic group compared to nondiabetics, as depicted in Table II. The gender distribution between diabetic and nondiabetic patients was significantly different (p=0.028). When the prognosis and survival rates were evaluated, 91.7% of the diabetic patients were discharged with complete recovery and 8.3% died, while these rates were 97.8% and 2.2% for nondiabetic patients, respec-

Table II. Comparison of gender, age, hospitalization status, outcome, and tomography findings for patients with and without diabetes.

		Non-DM (n=823)		DM (n=230)		*p*
		n	%	n	%	
Age, years, median (min-max)		42 (18-94)		65 (20-90)		**<0.001****
Gender	Female	355	43.1	118	51.3	**0.028***
	Male	468	56.9	112	48.7	
Hospitalization status	Ward	749	91.0	183	79.6	**<0.001***
	Intensive care	41	5.0	26	11.3	
	Deteriorating patients	33	4.0	21	9.1	
Survey	Recovering patients	805	97.8	211	91.7	**<0.001***
	Death	18	2.2	19	8.3	
Infiltration on thorax CT	No infiltration	234	28.4	38	16.5	**0.001***
	Confirmed infiltration	570	69.3	188	81.7	
	No CT	19	2.3	4	1.7	
Total		823	100.0	230	100.0	

*Chi-square test

Table III. Hematological parameters for patients with and without diabetes.

	Non-DM (n=823)	DM (n=230)	p
WBC (×10^9/L), median (min-max)	5.91 (1.35-52.68)	6.72 (2.6-24.04)	**<0.001****
NEU (×10^9 per L), median (min-max)	3.68 (0.02-50.47)	4.29 (1.28-65)	**<0.001****
NEU%, mean±SD	64.08±13.28	67.53±12.3	**<0.001***
LYM (×10^9/L), median (min-max)	1.4 (0.01-4.64)	1.41 (0.22-8.3)	0.384**
LYM%, mean±SD	25.4±11.33	22.07±10.33	<0.001*
NLR, median (min-max)	2.48 (0.01-68.58)	3.35 (0.83-38.11)	**<0.001****
LUCs (×10^9 per L), median (min-max)	0.1 (0.02-3.06)	0.11 (0.03-0.98)	**0.015****
LUCs%, median (min-max)	1.6 (0.2-61.5)	1.6 (0.4-5.2)	0.691**
Hb (g/dL), median (min-max)	14 (7.4-18.7)	13 (6.7-16.9)	**<0.001****
PLT, median (min-max)	226,000 (43,000-717,000)	227,000 (86,000-463,000)	0.286**

*Independent samples *t*-test
**Mann-Whitney U test

tively. The difference in mortality rates was statistically significant (p<0.001) between the groups. There were infiltrative chest tomography findings for 81.7% of the diabetic patients and 69.3% of the nondiabetic patients, and this difference was statistically significant (p=0.001) (Table II).

The mean WBC count, NEU count, NEU%, NLR, and LUC count were higher while Hb and LYM% were lower in diabetic patients compared to nondiabetic patients, and these differences were statistically significant (p<0.001, p<0.001, p<0.001, p<0.001, p=0.015, p<0.001, and p<0.001, respectively). There was no statistically significant difference in LYM number, %LUCs, or PLT count values between diabetic and nondiabetic patients (p=0.384, p=0.691, and p=0.286, respectively) (Table III).

The variables of age, HbA1c, and LYM had statistically significant impacts on disease severity in terms of the need for intensive care and mortality in the multivariate logistic regression model. Accordingly, a 1-unit increase in age significantly increased the risk of admission to the intensive care unit by 1.05 times (OR=1.053, 95% CI=1.008-1.099, p=0.020), a 1-unit increase in HbA1c values by 1.5 times (OR=1.561, 95% CI=1.146-2.124, p=0.005), and a 1-unit increase in LYM values by 0.24 times (OR=0.248, 95% CI=0.094-0.656, p=0.005). In other words, LYM count produced a value below 1, and the confidence interval was below 1 and did not include 1, indicating that LYM significantly reduced the risk of admission to the intensive care unit and was a protective factor. When the analyses were repeated by reversing the LYM values as 1/LYM, it was observed that a 1-unit increase in LYM levels significantly reduced the risk of admission to the intensive care unit by 7.5 times (OR=7.560, 95% CI=2.414-23.676, p=0.001) (Table IV).

When the LUC values of the patients hospitalized in the ward, followed in the intensive care unit, or transferred from the ward to the intensive care unit were compared separately according to the presence of diabetes, it was determined that the LUC counts did not create a statistically significant difference in patients with and without DM (p=0.214, p=0.224, and p=0.388, respectively).

Table IV. Multiple logistic regression analysis to determine risk factors affecting intensive care admission.

	B	SE	Exp(B)	95% CI	p
Age	0.052	0.022	1.053	1.008-1.100	**0.021**
HbA1c	0.477	0.158	1.612	1.182-2.198	**0.003**
1/LYM	2.023	0.582	7.560	2.414-23.676	**0.001**
Constant	-10.767	2.405	0.000		**<0.001**

*Multiple logistic regression analysis (backward LR)
Omnibus tests of model coefficient, <0.001
Nagelkerke R-square = 0.319
Hosmer and Lemeshow test = 0.605

Discussion

The present study has shown that LUC values in COVID-19 PCR-positive diabetic patients were significantly higher than those of nondiabetic patients. In the literature, there are very few studies examining %LUC. In a study of patients with invasive aspergillosis, %LUC was significantly higher, and a correlation was determined between %LUC and NLR[15]. The %LUC value was also significantly higher among chickenpox patients, and %LUC was considered to be beneficial in the differential diagnosis of varicella, herpes zoster, and Kaposi varicelliform eruptions, which can be challenging to diagnose, with sensitivity of 71.01% and specificity of 84.44%[16]. Vanker et al[17] demonstrated that %LUC values were significantly higher among HIV-positive patients. They suggested that LUCs may indicate virally activated lymphocytes. Individuals with chronic immune stimulation would have higher levels of circulating LUCs, and %LUCs may thus be used as a marker of progress in cases of HIV infection. Those authors also reported low levels of WBC count and high levels of CD38% on CD8 (an established marker of immune activation in HIV infection) in HIV-infected patients. Furthermore, there was an inverse correlation between CD4+ count (as an indicator of HIV disease progression) and %LUC, as well as a positive correlation between CD38% on CD8 and %LUC. In severe cases of COVID-19, low lymphocyte counts, high leukocyte counts, and high NLR values have been observed. The source of the cytokine storm in COVID-19 infections is hyperactive neutrophils and monocyte/macrophages[18]. Immune markers in the peripheral blood are seen to increase with the severity of COVID-19 infection. Neutrophil count, NLR, and neutrophil/CD8+ T-cell ratio all increase markedly, while total lymphocyte, CD8+, and CD4+ T-cell counts decrease[19]. CD4+ T-cells play a vital role in controlling pro-inflammatory and anti-inflammatory damage while supporting the inflammatory process in cases of Type 2 DM[20]. No study has been found in the literature to date examining the relationship between %LUC and CD8+ and CD4+ T-cells in patients with COVID-19. In patients with COVID-19, %LUC reduction and increases in D-dimer, NLR, and CRP have been identified as predictors of severe prognosis[21]. Our study did not reveal a relationship between LUCs, LUC% values, and surveillance in diabetic patients. However, we found the mean %LUC value to be significantly lower among intensive care patients compared to ward patients. Yan et al[22] in their study examining the surveillance of diabetic patients with severe COVID-19 infection, concluded that diabetes may cause an increased risk of death. They determined that WBC and neutrophil counts were higher and lymphocyte counts were lower among diabetic patients compared to nondiabetic patients. The present study has revealed that the WBC count, NEU count, and NLR were significantly higher among COVID-19-positive diabetic patients than nondiabetic patients. A population-based cohort study[23] from the United Kingdom reported that, with the emergence of COVID-19, there was a rapid and significant increase in mortality related to the cardiovascular and renal complications of diabetes, and, independently, glycemic control and body mass index. A meta-analysis[24] of 33 studies (16,003 patients) determined a 2-fold increase in mortality and risk of severe disease associated with COVID-19 in diabetic patients. Hyperglycemia contributes to the development of cytokine storms by increasing counterregulatory hormones, such as glucagon, cortisol, and epinephrine. It also increases pro-inflammatory cytokines, such as IL-6 and TNF-α[25]. The highest known death rate from COVID-19 has been indicated to be due to cardiometabolic diseases[26]. Knowing the role of endothelial dysfunction in the pathophysiology of COVID-19 in patients with cardiovascular and metabolic disorders may enable the development of new treatment strategies that will reduce the severity of infection[27]. Maiese et al[28] in their review, reported autopsy findings of deaths due to SARS-CoV-2, and they concluded that SARS-CoV-2 appears to cause endothelial dysfunction, which may be responsible for multiorgan dysfunction. A meta-analysis of 6 studies[29] in China that included 828 patients with COVID-19 concluded that high NLR values reflected an increased inflammatory process, possibly predicting poor prognosis. Circulating biomarkers are potential predictors of inflammation and immune status in the prognosis of COVID-19 patients, and previous studies[30] have confirmed that high NLR is an independent prognostic biomarker in cases of COVID-19. We hypothesize that the LUC parameter might be a potential biomarker in cases of COVID-19.

In our study, we found that the risk of hospitalization in the intensive care unit increased significantly with increases in HbA1c value and age, and the risk of hospitalization in the intensive care unit decreased significantly with a 1-unit increase in lymphocyte values. In their study that included 552 hospitals and the data of 1,099 patients in China, Guan et al[31] detected lymphocytopenia in 83.2% of all cases, thrombocytopenia in 36.2%, and leukopenia in 33.7%. A retrospective study[32]

of 500 patients confirmed that low lymphocyte and platelet counts were independent risk factors for mortality in hospitalized patients with COVID-19. Those authors developed a predictive algorithm (the BGM score) for mortality along with troponin I, age, D-dimer, and C-reactive protein, which were identified as other risk factors. They considered that this model, for which all variables of peripheral blood were obtained within the first 48 hours of admission, could support early interventions and enable early detection of patients at high risk of death. Merino et al[33] in their study in which peripheral blood samples were examined by flow cytometry, divided patients with COVID-19 into two groups as having atypical reactive lymphoid cells or not. They found low absolute neutrophil counts, high absolute lymphocyte counts, high Hb values, and high platelet counts in the reactive lymphocyte-positive group, with a significantly higher NLR in the lymphocyte-negative group and %LUC above 5% in the lymphocyte-positive group. That study revealed that the abundant production of reactive lymphocytes and virus-specific T-cells was associated with a good prognosis. In a study performed in Japan, patients with COVID-19 had atypical lymphocytes in their peripheral blood 1 week after the onset of the disease. These patients with atypical lymphocyte populations had a high prevalence of pneumonia, and two-thirds of the oxygen-treated patients had stable or improved clinical courses after the appearance of atypical lymphocytes[34]. Our study did not determine any difference in %LUC values between diabetic patients with and without lung involvement. An increase in LUCs may occur in leukemia due to blasts or in infectious mononucleosis due to an increase in atypical mononuclear cells. An increased LUCs population may also result from increased numbers of cytotoxic lymphocytes, natural killer cells, or reactive lymphoid cells[35]. Some studies[36] have reported sporadic observations of morphological changes in peripheral blood, such as reactive or plasmacytoid lymphocytes, abnormal monocytes/granulocytes/platelets, and leucoerythroblastic reactions in cases of COVID-19 infection.

Conclusions

Our study is the first in the literature to examine the LUC values of COVID-19-positive diabetic individuals, and that rate was found to be high. There are still unexplained points in the pathophysiology of COVID-19 and further studies are needed on these issues. Since novel discoveries mean new targets in treatment, we think that such studies are critical in the fight against SARS-CoV-2 in the current period, when the initial effects of the pandemic have decreased after vaccination studies, but new variants continue to emerge.

Conflict of Interest

The authors declare that they have no conflicts of interests.

Ethical Approval

This study was conducted in accordance with the principles of the Declaration of Helsinki. Ethics Committee Approval was obtained from Ankara City Hospital (Ethics Committee Approval No: E1-20-1107).

Funding

This research did not receive any financial support.

Authors' Contributions

Conception and design: Keskin M, Polat SB; Acquisition of data: Ates I, Izdes S, Güner HR; Analysis and interpretation of data: Topaloglu O; Drafting the article: Ersoy R; Supervision: Cakır B; Validation and final approval: all authors.

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