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Original article

Lymphopenia may be considered a marker for the need for invasive mechanical ventilation (IMV) in COVID-19 surviving patients

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ABSTRACT

Background: Although most studies describe the role of lymphocytes in COVID-19 severity and mortality, there is still a lack of data in the literature on whether lymphocytes may be good predictors of severity in surviving patients post-hospitalization for COVID-19. **Objectives:** This study aimed to investigate whether the lymphocyte count, measured at hospital admission, could be a potential marker of severity among COVID-19 survivor patients who needed hospitalization, and returned after one month of the discharge. **Methods:** Prospective observational cohort study of hospitalized unvaccinated adult patients diagnosed with COVID-19. Subjects underwent clinical and laboratory evaluation at the time of admission and after one month of discharge. **Results:** A total of 44 patients were included, 20 were admitted to the Intensive Care Unit and 10 required invasive mechanical ventilation (IMV). Patients with the lowest lymphocyte count values were predominantly male, had more ageusia, and needed more invasive mechanical ventilation. ROC curve analysis determined that lymphocyte count cutoff values ≤ 971 cell/mm3, area under the curve of 0.77 [CI: 0.61-0.92; p=0.01], sensitivity (80%), and specificity (61%) to identify the need for invasive mechanical ventilation. There was complete recovery of lymphocytes and no unfavorable outcome with a one-month follow-up after hospital discharge. **Conclusion:** Lymphocyte count ≤971 cells/mm3 had good accuracy in the prediction of the need for invasive mechanical ventilation in surviving patients hospitalized due to COVID-19.

Key-words: SARS-CoV-2; prognostic markers; lymphopenia; hospitalization; COVID-19.

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INTRODUCTION

Even with the large number of studies already published on Coronavirus disease 2019 (COVID-19)(1–10), there is still a lot of superficial and poorly defined information about the mechanisms causing the disease and a lack of knowledge about which patients may present serious complications during the hospital follow-up. Aspects such as age, comorbidities, and other risk factors are associated with greater severity and mortality in these patients, and it is well-recognized that lymphopenia may be a predictor of COVID-19 severity and mortality(1,2,11).

Lymphopenia is characteristic of other viral infections such as that caused by the Severe Acute Respiratory Syndrome Coronavirus 1 (SARS-CoV-1) and the Middle East Respiratory Syndrome Coronavirus (MERS-CoV)(12,13). Studies on severe acute respiratory syndrome (SARS) before the COVID-19 pandemic have suggested that the early and consistent lymphopenia found in these patients may be due to direct infection(12,13), destruction by other immune cells(13), sequestration of lymphocytes in tissues, cytokine-mediated cell death, as well as suppression of bone marrow or thymus for T cell generation(12). More recently, in a study on severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) infection, Shen et al. (2022)(3) deduced that SARS-CoV-2 infects T lymphocytes irrespective of angiotensin-converting enzyme 2 (ACE2), a transmembrane protein found in vascular endothelial cells, heart, kidneys, testicles and to a lesser extent the intestine and lungs(4,14) and one of the entry routes for the virus(5). Furthermore, its downregulation in SARS-CoV-2 participates in the development of severe disease(14,15). The study by Shen et al. (2022)(3) also suggests that infected lymphocytes not only lose the capacity to control the viral infection but can also transport the virus to other regions of the body through the blood flow, which may result in more serious infections in patients with COVID-19.

Although most studies describe the role of lymphocytes in SARS-COV-2 severity and mortality(1,2,6,16), there is still a lack of data in the literature on whether lymphocytes may be good predictors of severity in surviving patients during and post-hospitalization for COVID-19.

One of the resources adopted to treat the most serious forms of COVID-19 is the use of invasive mechanical ventilation (IMV), and the use of IMV influences the length of stay and morbidity and mortality of hospitalized patients (17,18). In a review and meta-analysis carried out by Chang et al. (2021) (19), 69% of all patients admitted to the intensive care unit (ICU) required IMV and, of these, 43% to 74% died. Such data make the identification of predictors of the need for IMV relevant.

This study aimed to investigate if the lymphocyte count, measured at hospital admission, could be a potential marker of IMV among COVID-19 survivor patients who needed hospitalization and returned one month after discharge for follow-up, died, or were readmitted in this period. Therefore, we hypothesize that survivor patients with lower lymphocyte count during hospitalization due to COVID-19 may present greater severity for the disease, as need of the ICU, IMV and longer hospital stay, and unfavorable outcomes during the one-month follow-up post-hospitalization, as death or re-hospitalization.

METHODS

Study design and setting

A prospective observational cohort study of adult patients diagnosed with COVID-19 admitted to the University Hospital at the Federal University of São Carlos (HU-UFSCar).

The study followed the guidelines established in the Declaration of Helsinki, and all procedures involving research participants were approved by the Research Ethics Committee of UFSCar (Number: 34344520.8.0000.5504).

According to addendum No. 5.572.107, approved by the Research Ethics Committee of the Federal University of São Carlos, there was an exemption from signing the Free and Informed Consent Form (FICF), and incorporation of a secrecy and confidentiality agreement in each patient's medical record due to the difficulty of contacting them and bringing them to the hospital after discharge.

Subjects

The study population included individuals aged ≥ 18 years old diagnosed with COVID-19 and not vaccinated, admitted to a university hospital from June/2020 to January/2021. All individuals had their diagnosis of COVID-19 confirmed by the Reverse transcription polymerase chain reaction (RT-PCR) technique at least 72 hours after the onset of symptoms. Exclusion criteria included a known diagnosis of interstitial pulmonary fibrosis or severe lung disease coursing with fibrosis on chest tomography; preceding diagnosis of heart failure with reduced left ventricular ejection fraction (LVEF <0.5) and/or previously known supraventricular or ventricular arrhythmias; patients coming from other health units with more than 24 hours of hospitalization; death during hospitalization, refusal to participate in the study and patients who did not attend the outpatient consultation after one month of discharge.

Procedure

At the time of hospital admission, the general clinical parameters were recorded; sociodemographic data, comorbidities, and risk factors through face-to-face interviews with patients or their families, in cases wherein patients were unable to respond (such information was validated and completed with data found in medical records). In the first 24 hours of admission, venous and arterial blood were collected for analysis of laboratory parameters. After inclusion in the research, a professional involved in the study followed the clinical evolution of each subject throughout hospitalization and return for outpatient follow-up after one month of discharge. Patients who died during hospitalization or did not attend the follow-up after discharge were excluded from this study.

Measurements

Clinical Assessment: at the time of admission, initial clinical parameters were obtained, such as heart rate (beats/minute), respiratory rate (breaths/minute), systolic and diastolic blood pressure (mmHg), peripheral oxygen saturation (%) and body temperature (°C). Other data were obtained through interviews: age (years); sex (male and female); the presence of symptoms (such as cough, dyspnea, fever, malaise, among others), and the time of onset of these symptoms at the time of admission; the presence of risk factors or previously known diseases (such as diabetes mellitus, systemic arterial hypertension, coronary artery disease, neoplasia, dyslipidemia, overweight or obesity) in addition to lifestyle habits such as alcoholism and smoking. Medication use, including name and daily dosage, was also noted.

In addition, a 12-lead electrocardiogram was performed at hospital admission and one month after discharge to assess the following parameters: rhythm disturbances (tachycardia heart rate (HR)>100bpm; bradycardia HR<50bpm; frequency and onset of supraventricular and ventricular extrasystoles; the presence of persistent or paroxysmal atrial fibrillation or flutter; the presence of monomorphic or polymorphic tachycardia); QT interval measurement (finding and corrected by HR); investigation of atrioventricular conduction disorder (first, second, and third-degree atrioventricular blocks); investigation of intraventricular conduction disorder (right and left bundle branch conduction disorders); the presence of atrial and ventricular overloads; ST segment analysis (presence of ST-segment elevation or depression; investigation of strain in left and right leads); investigation of S1Q3T3.

Hospitalization data: hospitalization data were also computed, such as total hospitalization time in days, need for hospitalization in the Intensive Care Unit and the length of stay in this unit, need or not IMV, and the time of IMV in days.

Laboratory tests: the laboratory tests were performed within the first 24 hours of hospitalization and with a one-month follow-up after discharge. The tests included complete blood count, arterial blood gases, sodium, potassium, creatinine, urea, D-dimer, troponin, C-reactive protein, and INR.

Follow-up: patients who survived returned for medical consultation one month after hospital discharge, where a new clinical evaluation, 12-lead electrocardiogram, and laboratory tests were performed.

Statistical Analysis

We used the test of Shapiro-Wilk test to verify the distribution of the data. Descriptive data was shown as a mean, standard deviation, and frequency (%). Student's t-test and Mann-Whitney test were used for normal and non-normal distributed data, respectively. All tests were made in Statistical Package for the Social Sciences (SPSS) and values were accepted as $P \leq 0.05$.

To determine the independent effect of lymphocyte count on the outcome, a multiple logistic regression analysis was performed using the need for IMV as the dependent variable and age, gender, Body Mass Index (BMI), and lymphocyte count at admission as independent variables.

Receiver operating characteristic (ROC) curve analysis: Cut-off points discriminated the precision of lymphocyte count in determining the need for IMV. The 95% confidence interval (CI) was used to determine the predictive ability of the clinical variables, with the lower limit being greater than 0.50. Subsequently, the cut-off points of the variables that obtained significant areas under the ROC curve were identified, with the respective sensitivity and specificity values.

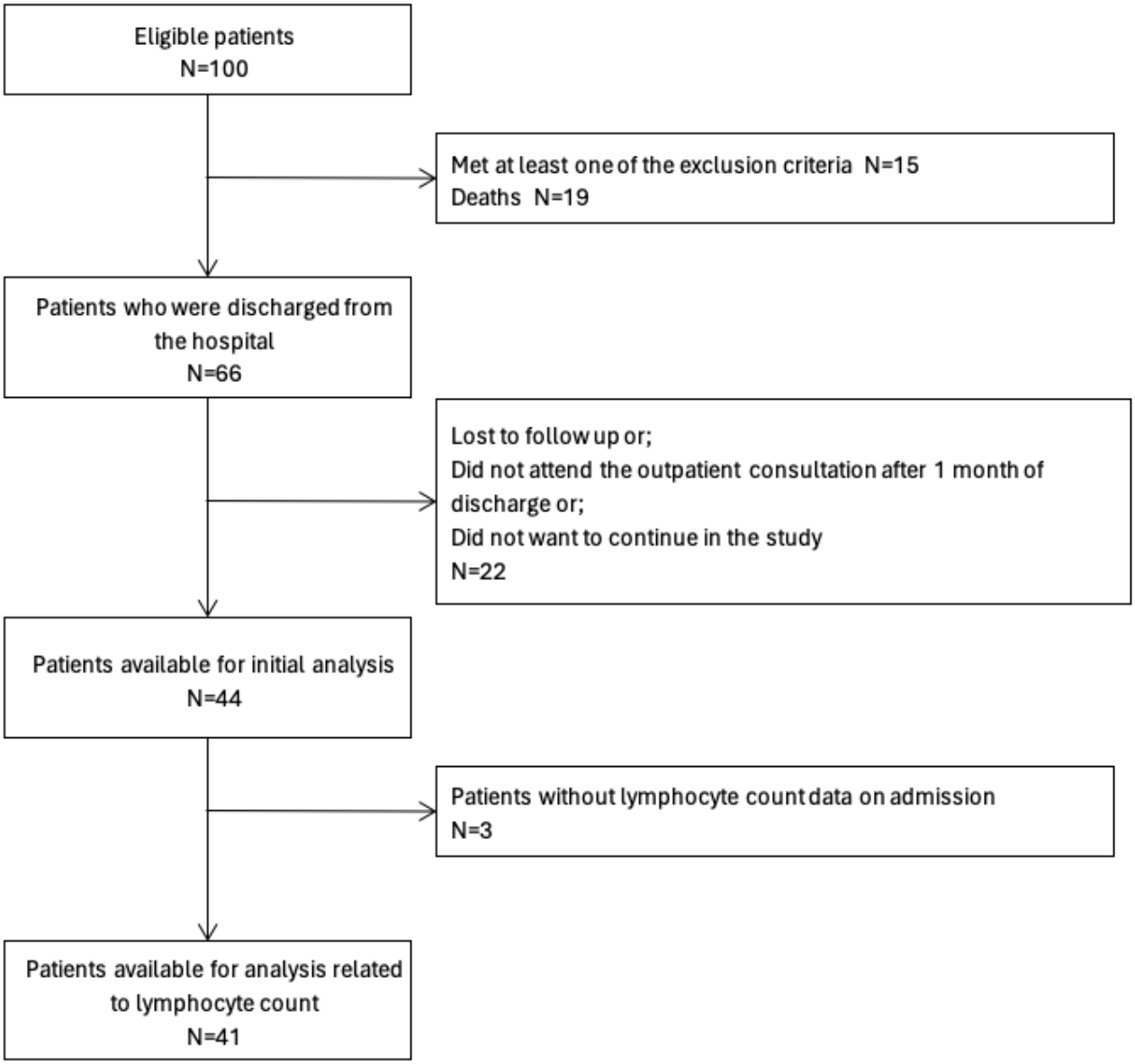
After the results found in the ROC curve analysis, we divided the patients into two groups taking into account the cutoff value found, and compared them using the Student's t-test for variables with normal distribution or Mann Whitney for non-parametric distribution, according to Shapiro-Wilks test, and significant values were accepted as $P \leq 0.05$.

RESULTS

Regarding the inclusion and exclusion criteria, 100 patients were admitted to the hospital during the study period, 19 died during the hospitalization, and 37 patients were excluded, because they met at least one of the exclusion criteria, or they

chose not to participate in the study anymore, or they did not attend the outpatient consultation after one month of discharge. Therefore, the sample initially considered for statistical analysis consisted of 44 patients. Of these 44 patients, after initial statistical analysis, three patients who did not have lymphocyte count data at admission were excluded, composing a final sample of 41 patients (Figure 1).

Figure 1. Flow diagram.



We evaluated 44 patients, aged 55±28 years, 29 men and 15 women, of whom 20 were admitted to the ICU and 10 required IMV. Most subjects were overweight/obese (77.3%) and the main risk factors were: smoking (36.4%), hypertension (SAH) (50%), diabetes mellitus type 2 (DM2) (29.5%), and coronary artery disease (CAD) (13.6%), as illustrated in Table 1. The main symptoms found on admission were dyspnea and cough (88.6% and 81.8%, respectively), followed by fever (65.9%) and malaise (56.8%), and the mean duration of symptoms at admission was 9 ± 5 days. The mean total length of hospital stay was 11±8 days (Table 1).

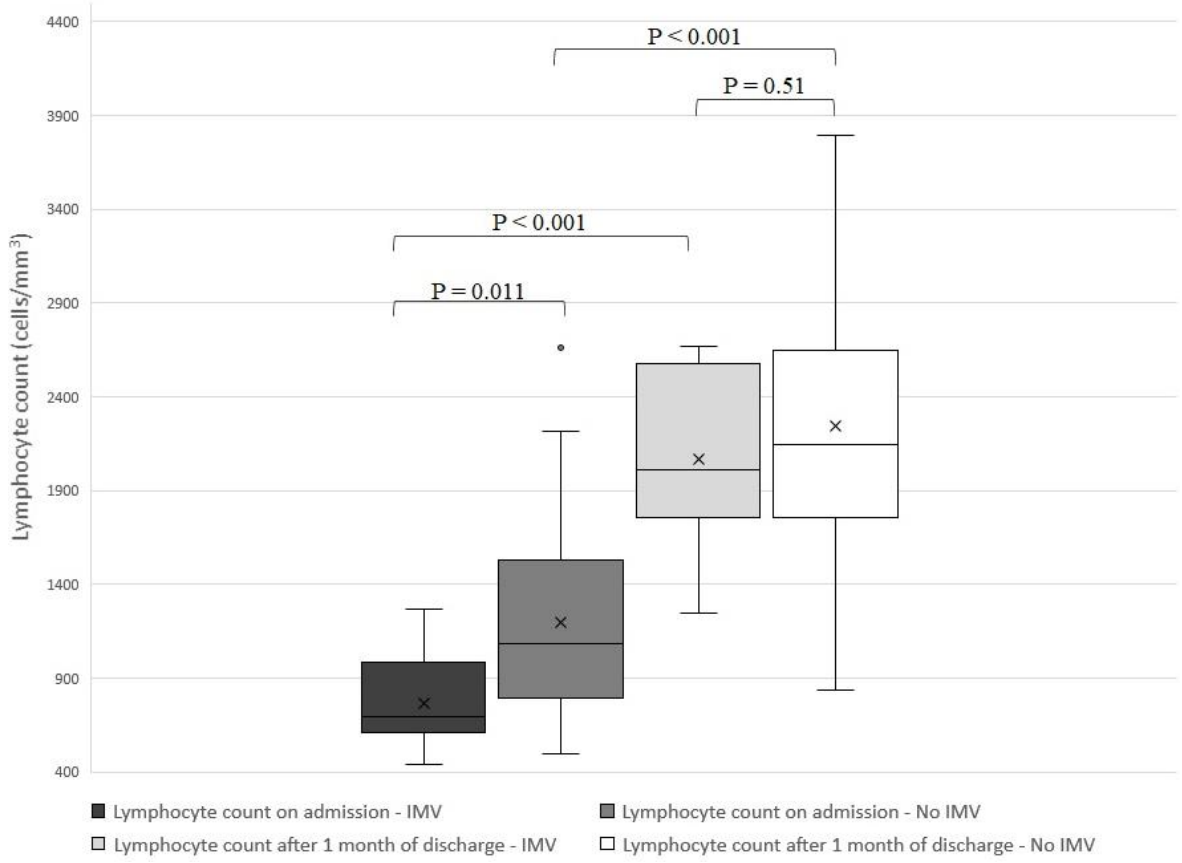
Table 1. Sample Characteristics.

Baseline Characteristics		
Variables		**Patients (n = 44)**
Age, years		54.45 ± 13.68
Gender	Male	29(65.91)
	Female	15(34.09)
BMI, Kg/m^2		30.25 ± 5.13
Comorbidities		
SAH		22(50.00)
Smoking		16(36.36)
DM2		13(29.55)
CAD		6(13.64)
Hospitalization data		
Length of hospitalization, days		10.48 ± 8.56
Patients admitted to ICU		20(45.45)
Length of stay in ICU, days		9.80 ± 7.40
Patients who required IMV		10(22.73)
Length of IMV, days		10.80 ± 6.46
Symptoms on admission		
Duration of symptoms at the time of admission, days		9.48 ± 5.26
Dyspnea		39(88.64)
Cough		36(81.82)
Fever		29(65.91)
Ageusia		8(18.18)
Clinical Parameters on Admission		
RR, breaths/min		24.84 ± 6.20
HR, beats/min		93.98 ± 22.43
SBP, mmHg		132.55 ± 28.62
Body temperature, °C		36.78 ± 0.95
SpO_2, %		90.41 ± 5.19

Values are presented as mean ± standard deviation for normally distributed variables or median and interquartile range for non-parametric variables or number (N), and percentage (%). BMI: Body Mass Index; SAH: Systemic Arterial Hypertension; DM2: Diabetes Mellitus type 2; CAD: Coronary Artery Disease; ICU: Intensive Care Unit; IMV: Invasive Mechanical Ventilation; RR: Respiratory rate; HR: Heart rate; SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure; SpO2: Peripheral oxygen saturation.

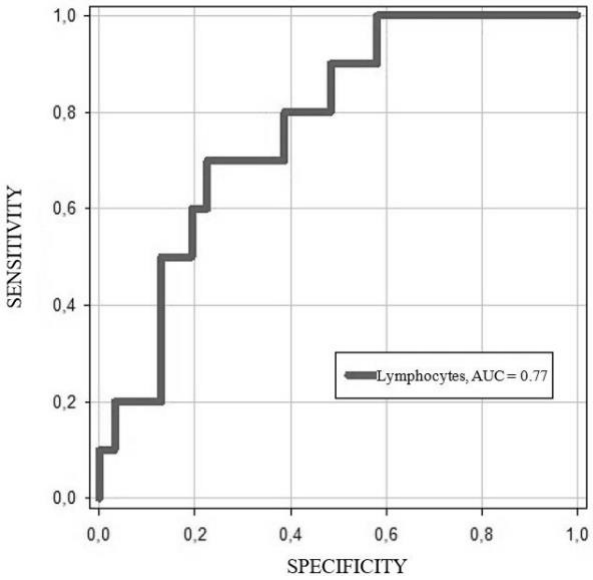
Figure 2 shows a correlation between lower lymphocyte count and the need for IMV (p=0.011) during hospitalization. This correlation was not observed in these patients at one-month follow-up after the discharge. Considering this correlation, there was performed a multiple logistic regression analysis, and the lymphocyte count was independently associated with the need for IMV (p=0.018) when considering other co-variates, such as age (p=0.33), gender (p=0.49), and BMI (p=0.18).

Figure 2. Lymphocyte count at admission and after one month of discharge: Comparison between the lymphocyte count of the group that required IMV and the group that did not need IMV at the time of admission and after one month of discharge.



Considering these findings, ROC curve analysis was performed to calculate a cut-off point for lymphocyte counts, and according to ROC analysis, cutoff values producing optimal sensitivity and specificity are sensitivity (80%) and specificity (61%), determining an ideal lymphocyte count ≤ 971 cell/mm3 to detect the need for IMV, as illustrated in Figure 3.

Figure 3. ROC curve: Area Under the Curve (AUC) 0.77 [CI: 0.61-0.92; p=0.01], sensitivity (80%) and specificity (61%), determining an ideal lymphocyte count > 971 cell/mm3 to detect the need for invasive mechanical ventilation.



After the finding of this cutoff point and excluding the three patients without lymphocyte count data on admission, the remaining 41 patients were divided into two groups: one with a lymphocyte count > 971 cells/mm3 and another with a lymphocyte count ≤ 971 cells/mm3. There was a significant association between lymphocyte count ≤ 971 cells/mm3 and male (p=0.031), presence of ageusia (p=0.017), and lower values of bicarbonate in arterial blood gases in the first 24 hours of hospitalization (p=0.042). The detailed results are described in Table 2.

After a one month of follow-up, patients with lymphocyte counts ≤ 971 cells/mm3 had complete recovery of lymphocyte values (2146 cells/mm3 [1754.75-2511.25]), showing no significant difference from those with lymphocyte counts > 971 cells/mm3. In the one-month follow-up, there was no death or re-hospitalization.

Table 1. Sample divided into groups according to lymphocyte value.

Data from the first 24 hours of hospitalization				
Variables		**Lymphocyte count**		
		> 971 cells/mm³ (n = 21)	**≤ 971 cells/mm³ (n = 20)**	***p***
Age, years		54.1±16.16	54.55±12.1	0.919
Gender	Male	10(22.72)	16(36.36)	**0.031***
	Female	11(25)	4(9.09)	
BMI, Kg/m²		31.18±6.6	29±3.23	0.213
Comorbidities				
SAH		11(25)	9(20.45)	0.636
DM2		7(15.91)	6(13.64)	0.819
CAD		3(6.82)	3(6.82)	0.948
Hospitalization data				
Duration of symptoms at the time of admission, days		9.9±6	8.6±3.7	0.414
Length of hospitalization, days		7.9±6.37	12.55±10.31	0.094
Patients admitted to ICU		7(15.91)	10(22.73)	0.279
Length of stay in ICU, days		7.57±8.86	11.9±7.04	0.279
Patients who required IMV		2(4.55)	8(18.18)	**0.023***
Length of IMV, days		15.5±9.19	9.88±6.05	0.397
Symptoms on admission, n(%)				
Dyspnea		19(43.18)	17(38.64)	0.592
Cough		17(38.64)	17(38.64)	NA
Fever		13(29.55)	14(31.82)	0.557
Ageusia		1(2.27)	7(15.91)	**0.017***
Clinical Parameters on Admission				
RR, breaths/min		24.95±7.03	25.25±5.68	0.883
HR, beats/min		94.71±14.83	93.65±29.95	0.885
SBP, mmHg		133.67±36.71	132.5±20.41	0.901
DBP, mmHg		80.81±22.75	82±11.79	0.836
Body temperature, °C		36.74±0.98	36.87±0.95	0.674
SpO_2, %		90.95±50.16	89.6±5.49	0.421
D-Dimer		1.188±1.235	1.157±1.088	0.934
Corrected QT Interval (Hodges)		528.91±48.16	551.47±40.38	0.27
At least 1 ECG change		9(20.45)	4(9.09)	0.251
Laboratory tests				
PH		7.45±0.05	7.45±0.03	0.738
PaO_2, mmHg		64.71±14.1	67.41±12.07	0.53
$PaCO_2$, mmHg		36.24±7.54	33.58±5.18	0.209
HCO^-_3, mmol/L		25.29±3.51	23.3±1.84	**0.042***

$SatO_2$, %	91.52±6.37	92.79±3.67	0.454
Urea, mg/dL	44.75±30.11	37.85±18.20	0.386
Creatinine, mg/dL	0.95±0.52	0.96±0.26	0.94
Hemoglobin, g/dL	14.16±1.76	14.24±2.08	0.891
Leukocyte count, cell/mm^3	10327.14±7888.35	6494±3410.49	0.052

Values are presented as mean ± standard deviation for normally distributed variables or median and interquartile range for non-parametric variables or number (N), and percentage (%). BMI: Body Mass Index; SAH: Systemic Arterial Hypertension; DM2: Diabetes Mellitus type 2; CAD: Coronary Artery Disease; ICU: Intensive Care Unit; IMV: Invasive Mechanical Ventilation; RR: Respiratory Rate; HR: Heart Rate; SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure; SpO_2: Peripheral Oxygen Saturation; ECG: electrocardiogram; PaO_2: Arterial Oxygen Tension; $PaCO_2$: Arterial CO_2 Tension; HCO^-_3: Bicarbonate; $SatO_2$: Arterial Oxygen Saturation; *: p-value less than 0.05; NA: No available.

DISCUSSION

In our study with survivor patients hospitalized due to COVID-19, including those who developed the critical form of the disease, we found that lymphopenia was an independent predictor for the need for IMV with a cutoff value for lymphocyte count ≤ 971 cell/mm3.

Several studies report lymphopenia as a marker of severity and mortality for COVID-19 patients(1,2,11). However, the influence of initial lymphocyte count in the clinical evolution of survivor patients is not well established. In addition, different from other papers that consider only the reference values of lymphocyte counts(1,2,6,11), this study defined a specific cutoff value as the predictor for the need for IMV in patients with COVID-19.

This study showed that lymphocyte values ≤ 971 cell/mm3 were more prevalent in males, in patients who presented ageusia, and lower values of bicarbonate in arterial blood gases on hospital admission. There was no direct relationship with age, as in the study performed by Ghizlane et al. (2021)(11). This alteration in the bicarbonate levels may be an indicator of the beginning of metabolic decompensation that would later contribute to IMV (Table 2). It is interesting to note that the lymphocyte count returned to normal during the one-month follow-up after the discharge and there was no difference between the groups, with no correlation with unfavorable outcomes in critical patients during the hospitalization (Figure 2). To explain the results found, it is necessary to understand the mechanism of entry of SARS-COV-2 into cells. One of the known entry pathways is through ACE2 receptors and transmembrane serine protease 2 (TMPRSS2)(5). In a simplified way, the viral spike protein (S) binds to the ACE2 receptor, and TMPRSS2 promotes virus entry into the cell(7,20). As stated earlier, several different tissues express ACE2 receptors(4,14), but for this study, we will focus on the airways and lungs.

The highest expression of ACE2 was observed in the nasal epithelium, followed by the ciliated epithelium of the conducting airways and to a lesser extent in alveolar type II cells(4,21). In the oral mucosa, the largest amount of ACE2 receptors is located on the tongue, mainly in taste cells(8), which could explain ageusia in COVID-19 patients if these cells were infected by the virus(8,9).

In parallel, Okwan-Duodu et al. (2021)(7), in a study about TMPRSS2, observed that the expression of TMPRSS2 in males is significantly higher, and that type II alveolar cells that express both ACE2 and TMPRSS2 are 3 times higher in males, which may provide the basis to explain the greater severity of COVID-19 in male(7) and the greater correlation between the need for IMV in males found in this study.

Regarding lymphocytes, Shen et al. (2022)(3), demonstrated that T lymphocytes, which in most cases lack ACE2 receptors, are directly infected by SARS-COV-2 independently of ACE2, possibly through leukocyte-associated molecule-1 (LFA-1) but the mechanism has not yet been well described.

Another important finding in our study is the prevalence of patients with a BMI ≥ 25 kg/m2 who required hospitalization. Keller et al. (2022)(10) also observed that obese patients hospitalized for COVID-19 had greater severity, in addition to having more comorbidities and mortality. Study limitations: the small number of participants due to organizational changes in the admission of COVID-19 patients by the hospital during the study, the lack of analysis of data such as oxygen use and non-invasive ventilation, and the non-attendance to outpatient consultations within one month of hospital discharge.

Despite the limitations, the results found in this study were important for a more complete and detailed knowledge of the natural evolution of COVID-19 in cases of unvaccinated patients and provide new information about predictors of severity at

the time of hospital admission. Similar studies with a larger sample size and the inclusion of patients who died are worthy of definitive conclusions.

CONCLUSION

Lymphocyte count ≤971 cells/mm3 had good accuracy in the prediction of the need for invasive mechanical ventilation in survivor patients hospitalized due to COVID-19. After one month of follow-up, in this limited sample, there was a complete recovery of lymphocyte count without unfavorable outcomes in these patients.

REFERENCES

1. Huang I, Pranata R. Lymphopenia in severe coronavirus disease-2019 (COVID-19): systematic review and meta-analysis. J Intensive Care. 2020 Dec 24;8(1):36.
2. Toori KU, Qureshi MA, Chaudhry A. Lymphopenia: A useful predictor of COVID-19 disease severity and mortality. Pak J Med Sci. 2021 Sep 6;37(7).
3. Shen XR, Geng R, Li Q, Chen Y, Li SF, Wang Q, et al. ACE2-independent infection of T lymphocytes by SARS-CoV-2. Signal Transduct Target Ther. 2022 Dec 11;7(1):83.
4. Heijink IH, Hackett T, Pouwels SD. Effects of cigarette smoking on SARS-CoV-2 receptor ACE2 expression in the respiratory epithelium. J Pathol. 2021 Apr 26;253(4):351–4.
5. Leng Z, Zhu R, Hou W, Feng Y, Yang Y, Han Q, et al. Transplantation of ACE2- Mesenchymal Stem Cells Improves the Outcome of Patients with COVID-19 Pneumonia. Aging Dis. 2020;11(2):216.
6. Waikar C, Gour V, Pranay L, Hingwe S. Lymphocyte count and A-DROP score in COVID-19 patients: A retrospective observational study. Journal of Acute Disease. 2022;11(3):115.
7. Okwan-Duodu D, Lim EC, You S, Engman DM. TMPRSS2 activity may mediate sex differences in COVID-19 severity. Signal Transduct Target Ther. 2021 Dec 1;6(1):100.
8. Xu H, Zhong L, Deng J, Peng J, Dan H, Zeng X, et al. High expression of ACE2 receptor of 2019-nCoV on the epithelial cells of oral mucosa. Int J Oral Sci. 2020 Dec 24;12(1):8.
9. Tanasa I, Manciuc C, Carauleanu A, Navolan D, Bohiltea R, Nemescu D. Anosmia and ageusia associated with coronavirus infection (COVID 19) - what is known? Exp Ther Med. 2020 May 28;
10. Keller K, Sagoschen I, Schmitt VH, Sivanathan V, Espinola-Klein C, Lavie CJ, et al. Obesity and Its Impact on Adverse In-Hospital Outcomes in Hospitalized Patients With COVID-19. Front Endocrinol (Lausanne). 2022 May 2;13.
11. Ghizlane EA, Manal M, Abderrahim EK, Abdelilah E, Mohammed M, Rajae A, et al. Lymphopenia in Covid-19: A single center retrospective study of 589 cases. Annals of Medicine and Surgery. 2021 Sep;69:102816.
12. Chu H, Zhou J, Wong BHY, Li C, Chan JFW, Cheng ZS, et al. Middle East Respiratory Syndrome Coronavirus Efficiently Infects Human Primary T Lymphocytes and Activates the Extrinsic and Intrinsic Apoptosis Pathways. Journal of Infectious Diseases. 2016 Mar 15;213(6):904–14.
13. Gu J, Gong E, Zhang B, Zheng J, Gao Z, Zhong Y, et al. Multiple organ infection and the pathogenesis of SARS. Journal of Experimental Medicine. 2005 Aug 1;202(3):415–24.
14. Imai Y, Kuba K, Ohto-Nakanishi T, Penninger JM. Angiotensin-Converting Enzyme 2 (ACE2) in Disease Pathogenesis. Circulation Journal. 2010;74(3):405–10.
15. Jia H. Pulmonary Angiotensin-Converting Enzyme 2 (ACE2) and Inflammatory Lung Disease. Shock. 2016 Sep;46(3):239–48.
16. Yang X, Yu Y, Xu J, Shu H, Xia J, Liu H, et al. Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study. Lancet Respir Med. 2020 May;8(5):475–81.
17. Lentz S, Roginski MA, Montrief T, Ramzy M, Gottlieb M, Long B. Initial emergency department mechanical ventilation strategies for COVID-19 hypoxemic respiratory failure and ARDS. Am J Emerg Med. 2020 Oct;38(10):2194–202.
18. Gosangi B, Rubinowitz AN, Irugu D, Gange C, Bader A, Cortopassi I. COVID-19 ARDS: a review of imaging features and overview of mechanical ventilation and its complications. Emerg Radiol. 2022 Feb 26;29(1):23–34.
19. Chang R, Elhusseiny KM, Yeh YC, Sun WZ. COVID-19 ICU and mechanical ventilation patient characteristics and outcomes—A systematic review and meta-analysis. PLoS One. 2021 Feb 11;16(2):e0246318.
20. Hoffmann M, Kleine-Weber H, Schroeder S, Krüger N, Herrler T, Erichsen S, et al. SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. Cell. 2020 Apr;181(2):271-280.e8.
21. Hou YJ, Okuda K, Edwards CE, Martinez DR, Asakura T, Dinnon KH, et al. SARS-CoV-2 Reverse Genetics Reveals a Variable Infection Gradient in the Respiratory Tract. Cell. 2020 Jul;182(2):429-446.e14.