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HEALTH AND REHABILITATION

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FREQUENCY PF LYMPHOPENIA IN MODERATE TO SEVERE COVID 19 PATIENTS

Original Research

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Conflict of Interest: None
Grant Support & Financial Support: None

ABSTRACT

Background: Lymphopenia, a decrease in lymphocyte count, is frequently observed in severe COVID-19 cases and may indicate disease severity. This retrospective study aimed to identify factors predictive of lymphopenia, evaluate its relationship with COVID-19 severity, and assess its impact on mortality. Understanding lymphopenia's role could enhance clinical assessment and management of high-risk patients, particularly those with pre-existing comorbidities.

Objective: To analyze the predictive factors for lymphopenia, establish its association with COVID-19 severity, and examine its effect on patient outcomes, including mortality.

Methods: This retrospective, single-center observational study included 500 COVID-19 patients admitted to the Intensive Care Unit (ICU) between 2020 and 2023. Patients were divided into two groups based on lymphocyte counts: 300 patients (60%) with lymphopenia and 200 (40%) without. Demographic, clinical, and laboratory data, including CT scan findings and inflammatory markers, were collected. Statistical comparisons were made to identify differences between groups, and key outcomes such as mortality, ICU interventions, and length of hospital stay were evaluated.

Results: Of the 500 patients, the lymphopenia group had a median age of 65 years, significantly higher than the 62 years in the non-lymphopenia group. Lymphopenia patients had higher rates of diabetes and hypertension. Elevated levels of CRP (190.27 mg/L) and LDH (583 U/L) were noted in the lymphopenia group, with a significant association between lymphopenia and CT scan severity. While lymphopenia patients required more non-invasive ventilation (110 cases) and invasive ventilation (150 cases), lymphopenia also correlated with a higher mortality rate (100 vs. 20 in non-lymphopenia).

Conclusion: Lymphopenia is prevalent in severe COVID-19, particularly among older adults and patients with comorbidities, and serves as a potential marker for adverse clinical outcomes. Further studies are needed to establish lymphopenia's predictive role in COVID-19 severity and prognosis, supporting its inclusion in clinical assessments.

Keywords: COVID-19, lymphocyte count, lymphopenia, mortality, predictive markers, severity, ventilation.

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INTRODUCTION

In December 2019, a novel disease known as COVID-19 emerged in Wuhan, Hubei Province, China, rapidly becoming a global pandemic and causing widespread morbidity and mortality. COVID-19 primarily affects the respiratory system, with acute respiratory failure as its most severe manifestation, frequently necessitating oxygen supplementation and occasionally progressing to multiorgan failure or death (1). As the disease was new, unique clinical management guidelines and diagnostic measures were developed to improve patient outcomes. Among various prognostic factors, lymphopenia has emerged as a critical indicator of disease severity and hospitalization risk in COVID-19 patients. Lymphocytes play an essential role in maintaining immunological balance and regulating the body's inflammatory response, making lymphopenia a reliable marker for determining the degree of immune compromise in these patients.

Research has proposed several potential mechanisms for lymphocyte depletion in COVID-19. One hypothesis suggests that the virus might directly infect lymphocytes due to their expression of the ACE2 receptor, making them a direct target for the viral invasion (2). Another theory proposes that the virus may directly damage lymphoid organs, such as the spleen and thymus, potentially leading to significant lymphocyte loss. This organ-specific damage could be linked to lymphocytic dysfunction, and further pathological studies may substantiate this hypothesis in the future. Additionally, lymphocyte death might be influenced by persistent inflammatory cytokine disruption. Studies show that pro-inflammatory cytokines, including tumor necrosis factor α and interleukin-6, contribute to lymphocyte depletion (3). Lastly, metabolic disturbances, such as elevated blood lactic acid levels frequently seen in severe COVID-19 cases, might inhibit lymphocyte proliferation, contributing to immunosuppression (4).

Supporting evidence includes a study by R.S.M. Wong et al. (2023), which found that 98% of COVID-19 cases in Hong Kong exhibited lymphopenia (5). Other studies highlight the utility of absolute lymphocyte count (ALC) in emergency departments for differentiating COVID-19 patients from those with other conditions, facilitating more precise isolation measures and reducing exposure risk for healthcare workers (6). Early identification and appropriate isolation of suspected COVID-19 patients using predictive parameters like lymphopenia can also reduce resource utilization, lowering hospital costs and minimizing the consumption of personal protective equipment. Lymphopenia has been associated with a range of clinical symptoms in COVID-19, such as fever, dyspnea, cough, and asthenia, which may precede organ failure. Research shows that patients with comorbidities and severe lymphopenia exhibit higher mortality rates. Predictive markers, including inflammatory indicators such as lactate dehydrogenase, interleukin-6, ferritin, fibrinogen, C-reactive protein (CRP), and the degree of pulmonary damage visible on CT scans, help clinicians evaluate COVID-19 severity (7). These findings underscore the importance of lymphopenia as a key marker in clinical assessments and may support incorporating total lymphocyte count into routine testing upon hospital admission. This study aims to retrospectively analyze the frequency and association of lymphopenia with the severity of COVID-19 infection. It seeks to identify predictive factors linked to lymphopenia and assess its relevance to patient mortality, ultimately evaluating whether lymphocyte count should become a standard part of COVID-19 admission protocols.

METHODS

This retrospective, single-center observational study was conducted in a hospital-based Intensive Care Unit (ICU) and included patients with clinical, biological, and/or radiological evidence of COVID-19. Patients admitted to the ICU for COVID-19-related acute respiratory failure between 2020 and 2023 were evaluated. Data were gathered from electronic medical records for a cohort of 500 patients, who were divided into two groups based on the presence of lymphopenia, defined as an absolute lymphocyte count (ALC) of less than 1.0×10^9/L. Clinical, laboratory, treatment, and outcome data were compared between the lymphopenic and non-lymphopenic groups. All patients underwent computed tomography (CT) scans to assess COVID-19-related lung damage. Based on scanner impairment, lung damage was classified into severe (50–100% impairment) and non-severe (10–50% impairment) categories. Demographic and clinical data collected included age, gender, race/ethnicity, and relevant comorbid conditions such as obesity (body mass index ≥30 kg/m²), smoking status, hypertension, diabetes, coronary heart disease, chronic kidney disease, cancer, and chronic obstructive pulmonary disease (COPD). Laboratory values, including ALC, creatinine, lactate dehydrogenase (LDH), interleukin-6 (IL-

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6), D-dimers, and C-reactive protein (CRP), were documented within 24 hours of hospitalization. Patients were monitored from ICU admission until either hospital discharge or death. In cases where patients were readmitted during the study period, the most severe outcome was recorded as the primary endpoint.

The primary outcome measure was all-cause mortality during hospitalization. Secondary outcomes included the use of invasive mechanical ventilation, the requirement for dialysis due to acute kidney injury (AKI), and ICU admission necessity. The study's inclusion criteria encompassed adult patients (aged 18 years or older) admitted to the ICU from 2020 to 2023, those in the post-infectious phase with positive COVID-19 serology, and patients requiring oxygen therapy due to oxygen saturation below 92%. Additional risk factors for inclusion included COPD, obesity, diabetes, and smoking status among patients presenting with respiratory symptoms. Exclusion criteria included ICU admissions of less than 24 hours, patients with pre-existing lymphopenia from other pathologies, individuals younger than 18 years, missing ALC data, and patients with known HIV infection or hematologic disease. Statistical analyses were performed using OpenEpi software. Qualitative variables were presented as counts and percentages, while quantitative variables were expressed as mean ± standard deviation (SD) or median with interquartile range (IQR). A univariate analysis was conducted to compare the lymphopenic and non-lymphopenic patient groups. Visual representations, including tables, graphs, and pie charts, were created using Microsoft Excel to illustrate findings.

RESULTS

Five hundred patients admitted to the ICU for COVID-19-related acute respiratory failure were included in the study. Among these, 300 patients (60%) presented with lymphopenia at admission, while the remaining 200 (40%) maintained normal lymphocyte counts. The median age of patients with lymphopenia was 65 years, significantly higher than the median age of 62 in those without lymphopenia ($P < 0.0001$). Gender distribution revealed a marked male predominance in the lymphopenia group, with 74.2% being male compared to 55.6% in the non-lymphopenia group, also statistically significant ($P < 0.0001$). No significant difference in body mass index (BMI) was noted between the groups, with a median BMI of 26 kg/m² in both.

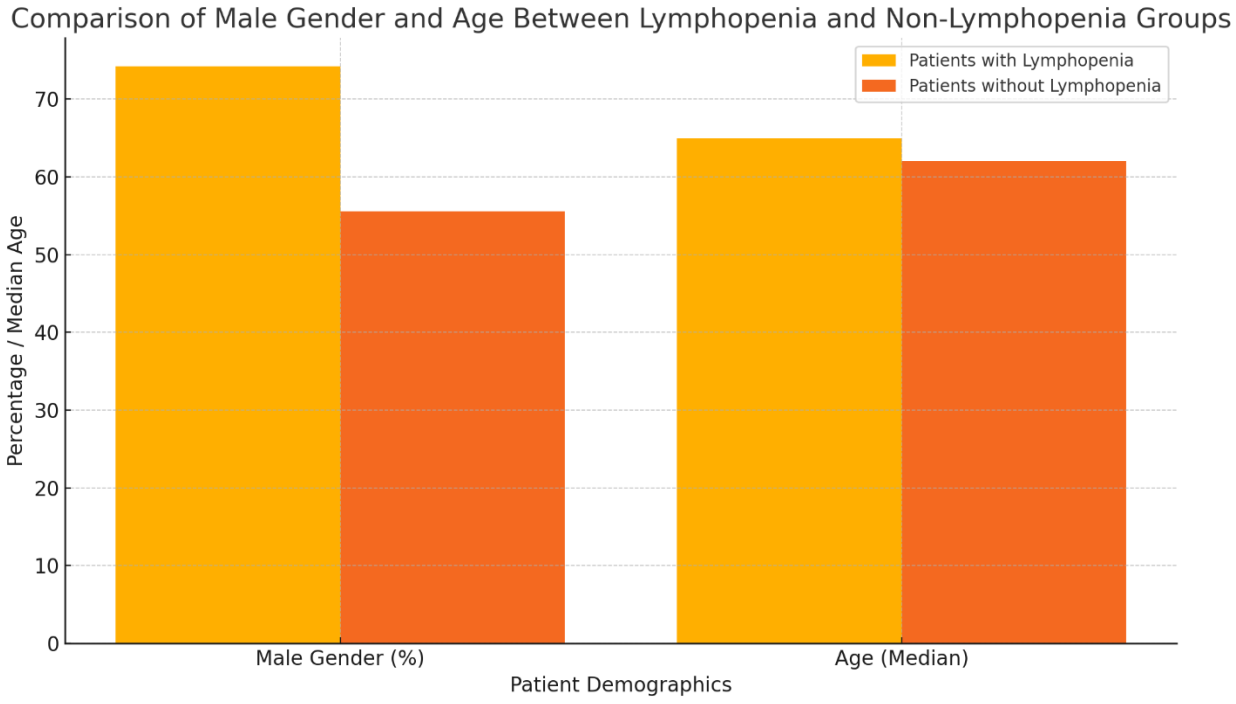

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Table 1: Patients Demographic Data

Patients' parameters	Patients with lymphopenia, n= 300	Patients without lymphopenia, n= 200
Male gender	225 (75%)	110 (55%)
Age	65	62
BMI	26	26

Laboratory analyses highlighted that patients with lymphopenia had elevated white blood cell (WBC) counts, with a median WBC count of 11.10 x10⁹/L (IQR 7.78–14.87), compared to a median of 9.66 x10⁹/L (IQR 6.71–15.12) in the non-lymphopenia group. Platelet counts were slightly lower in the lymphopenic group, with a median of 232.0 x10⁹/L (IQR 168.5–304.0), versus 246.0 x10⁹/L (IQR 180.0–335.0) in the non-lymphopenic group. Levels of lactate dehydrogenase (LDH) were notably elevated in the lymphopenia group, with a median of 583.0 U/L (IQR 397.5–825.5), compared to 435.0 U/L (IQR 253.0–827.5) in patients without lymphopenia. C-reactive protein (CRP) was markedly higher in the lymphopenia group, with an average of 190.27 mg/L versus 146.0 mg/L in the non-lymphopenia group, indicating a stronger inflammatory response among patients with lymphopenia.

Table 2: Laboratory Parameters Of The Patients

Laboratory parameters	Patients with lymphopenia	Patients without lymphopenia
White blood cell (x109/L)	11.10 (7.78–14.87)	l9.66 (6.71–15.12) 0
Platelets (x109/L)	232.0 (168.5–304.0)	246.0 (180.0–335.0)
LDH (U/L)	583.0 (397.5–825.5)	435.0 (253.0–827.5)
CRP (mg/L)	190.27	146.0

Prognosis and outcome measures demonstrated a higher incidence of severe interventions among lymphopenic patients. High-concentration oxygen masks were used in 250 patients with lymphopenia, as opposed to 100 patients without. Non-invasive ventilation was required in 110 patients in the lymphopenia group compared to 30 in the non-lymphopenia group. Additionally, invasive mechanical ventilation was more frequently needed among lymphopenic patients, with 150 requiring this intervention compared to 62 in the non-lymphopenia cohort. The median hospital stay duration remained the same for both groups at seven days. Survival outcomes showed that 200 patients in the lymphopenia group improved and were discharged, while 100 patients succumbed to the disease. In contrast, 180 patients without lymphopenia were discharged, and only 20 patients died, suggesting a substantially higher mortality rate in the lymphopenic group.

Table 3: Prognosis of the Patients

prognosis	Patients with lymphopenia n-300	patients without lymphopenia n- 200
High concentration masks use	250	100
Noninvasive ventilation	110	30
Invasive ventilation	150	62
Duration of hospital stay	7	7
Improved and discharged patients	200	180
Death	100	20

Figure 1 Incidence of hospital deaths & mechanical ventilation in the both groups

A closer analysis of absolute lymphocyte counts (ALCs) revealed that lower ALCs were strongly associated with adverse clinical outcomes, including mortality, ICU admission, and the need for invasive mechanical ventilation ($P < 0.001$, Mann-Whitney U-test). This relationship indicates that lymphopenia on admission may serve as a predictor of poor prognosis in COVID-19 patients.

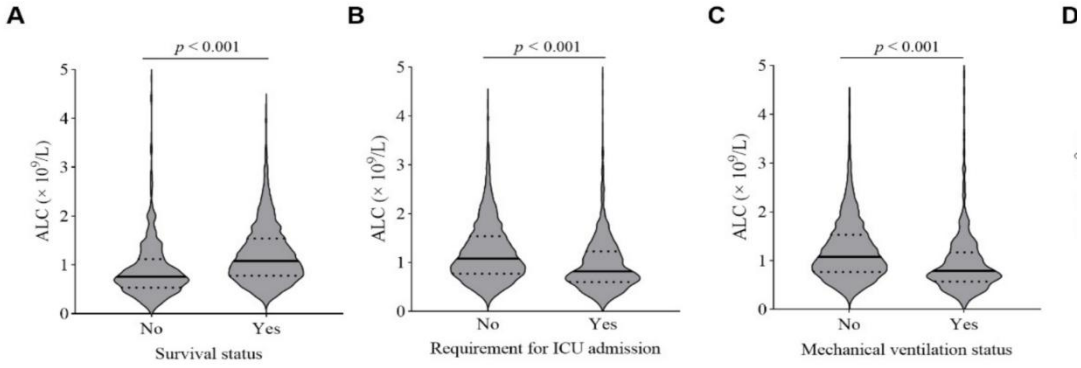

Figure 2 Relationships of ALCs

Relationships of ALCs on hospital admission with adverse clinical outcomes in patients with COVID-19. Violin plots of absolute lymphocyte counts (ALCs) showing significantly lower lymphocyte counts in patients who died (A) and those who required ICU admission (B), invasive mechanical ventilation (C), than those who were not ($p < 0.001$, Mann-Whitney U-test). Solid black lines indicate medians, and dashed black lines represent quartile ranges. A comparison of comorbid conditions between patients with and without lymphopenia provided further insight into the role of underlying health factors in COVID-19 severity. Common comorbidities, including obesity, hypertension, diabetes, coronary heart disease, chronic kidney disease, and COPD, were more prevalent among patients with lymphopenia. These associations suggest that lymphopenia, coupled with existing comorbidities, may serve as a critical predictor of adverse outcomes in COVID-19, highlighting the importance of comprehensive patient assessments upon ICU admission. This analysis reinforces the need for targeted interventions in high-risk groups to mitigate severe clinical trajectories and improve overall prognosis.

DISCUSSION

This study's findings demonstrate a significant association between lymphopenia and epidemiological traits, comorbidities, inflammatory biomarkers, and clinical outcomes in COVID-19 patients. Lymphopenia, observed in 60% of patients upon ICU admission, correlated with severe disease presentations, extended hospital stays, and elevated mortality rates. These results align with previous studies, such as Liu Y et al. in Shenzhen, China, which similarly indicated that lymphopenia is a robust indicator of COVID-19 severity (11). This observation also finds parallels with other viral infections, including SARS, MERS, and respiratory syncytial virus, where lymphopenia was a key predictor of disease severity (8, 9, 10). The pathophysiology underlying lymphopenia in COVID-19 may be linked to the cytokine storm, where unrestrained hypercytokinemia exacerbates viral load and immune exhaustion. COVID-19's impact on ACE2 receptors, which facilitate the virus's entry into T lymphocytes and other cells, potentially explains the depletion of lymphocytes in severe cases (12). This inflammatory cytokine storm, in turn, contributes to multi-organ failure and is strongly associated with poor outcomes. Additionally, this study's findings revealed that laboratory markers of inflammation, such as elevated CRP and

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LDH levels, were closely associated with lymphopenia, reinforcing the relationship between immune dysregulation and severe COVID-19.

Our analysis identified that lymphopenia was more common among older adults and males, a finding consistent with studies like Tavakolpour et al. (14). Patients with diabetes also showed higher rates of lymphopenia, highlighting the interplay between chronic conditions and immune vulnerability in COVID-19. The lack of fever in nearly 20% of lymphopenic patients emphasizes the need for vigilance in COVID-19 assessment, as asymptomatic presentations can obscure the disease's progression and hinder effective pandemic control. These results mirror Jiheng Liu et al.'s study, which reported similar findings, underscoring the importance of screening even mild cases for lymphopenia (15). Furthermore, this study corroborated the relationship between lymphopenia and CT scan abnormalities. Severe lung impairment on CT scans was strongly associated with lymphopenia, suggesting that lymphocyte counts could serve as an efficient classification tool for assessing COVID-19 severity, as noted in Tan et al.'s research (16). Our findings also support the prognostic utility of lymphopenia in predicting mortality, with evidence that lymphocyte levels recover before other clinical markers, potentially serving as an early indicator of recovery.

While these findings contribute to understanding the predictive role of lymphopenia in COVID-19, the study's retrospective design imposed limitations, such as incomplete data on biological tests, CT scans, therapies, and outcomes due to the retrospective nature of file collection. This limitation restricts the ability to establish causative relationships and predict the illness's trajectory accurately. Future studies should consider a prospective design to strengthen the evidence for lymphopenia's role in disease stratification and explore risk factors associated with lymphopenia in depth. Further investigation into lymphocyte dynamics throughout COVID-19 progression could enhance clinical strategies, particularly for assessing the severity of acute respiratory distress syndrome (ARDS) in COVID-19 patients. This study underscores the significance of lymphopenia as a predictor of adverse outcomes in COVID-19. By highlighting the correlation between lymphopenia and critical indicators, such as inflammatory biomarkers, demographic factors, and radiographic findings, these findings advocate for the early identification of lymphopenia in high-risk patients, potentially guiding targeted interventions and improving clinical outcomes.

CONCLUSION

Lymphopenia emerged as a prominent feature among severe COVID-19 patients, particularly in those with advanced age and underlying comorbidities, underscoring its potential as a marker of disease severity. This study reinforces the relevance of monitoring lymphocyte levels as an integral part of assessing COVID-19 prognosis, given its association with adverse clinical outcomes, including mortality. Although further studies are necessary to solidify lymphopenia's role in predicting disease severity, these findings highlight its importance as a clinical indicator, suggesting that early identification of lymphopenia could improve patient management and outcomes in COVID-19.

REFERENCES

1. Chen N, Zhou M, Dong X, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus in Wuhan, China: a descriptive study. Lancet. 2020;395(10223):507-513.

2. Xu H, High expression of ACE2 receptor of 2019-nCoV on the epithelial cells of oral mucosa. Int J Oral Sci. 2020;12(8).

3. Liao YC, IL-19 induces production of IL-6 and TNF-alpha and results in cell apoptosis through TNF-alpha. J Immunol. 2002;169:4288-97.

4. Huang C, Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet. 2020;395(10223):497-506.

5. Ahmed S, Ahmad QM, Akhtar T, Usman M, Al Rehman S, Ayub MR. Incidence of Rifampicin-Resistant Strains in Pediatric Cases Newly Identified with Pulmonary Tuberculosis. Annals of Punjab Medical College. 2023 Dec 31;17(4):440-5. https://doi.org/10.29054/apmc/2023.1306

6. 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;46:16-9.

7. Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet. 2020;395(10223):497-506.

8. Booth CM, Matukas LM, Tomlinson GA, et al. Clinical features and short-term outcomes of 144 patients with SARS in the Greater Toronto Area. JAMA. 2003;289(21):2801-9.

9. Arabi YM, Arifi AA, Balkhy HH, et al. Clinical course and outcomes of critically ill patients with Middle East respiratory syndrome coronavirus infection. Ann Intern Med. 2014;160:389-97.

10. O'Donnell DR, Carrington D. Peripheral blood lymphopenia and neutrophilia in children with severe respiratory syncytial virus disease. Pediatr Pulmonol. 2002;34:128-30.

11. Liu Y, Yang Y, Zhang C, et al. Clinical and biochemical indexes from 2019-nCoV infected patients linked to viral loads and lung injury. Sci China Life Sci. 2020;63(3):364-74.

12. Cameron MJ, Ran L, Xu L, et al. Interferon-mediated immunopathological events are associated with atypical innate and adaptive immune responses in patients with severe acute respiratory syndrome. J Virol. 2007;81(16):8692-706.

13. Lee N, Hui D, Wu A, et al. A major outbreak of severe acute respiratory syndrome in Hong Kong. N Engl J Med. 2003;348(20):1986-94.

14. Tavakolpour S, Taha R, Wei EX, Rhashidian M. Lymphopenia during the COVID-19 infection: what it shows and what can be learned. Immunol Lett. 2020;225:31-2.

15. Liu J, Li H, Luo M, et al. Lymphopenia predicted illness severity and recovery in patients with COVID-19: a single-center, retrospective study. PLoS One. 2020;15(11).

16. Tan L, Wang Q, Zhang D, et al. Lymphopenia predicts disease severity of COVID-19: a descriptive and predictive.

17. Zheng M, Gao Y, Wang G, et al. Functional exhaustion of antiviral lymphocytes in COVID-19 patients. Cell Mol Immunol. 2020;17:533-5.