Open Access

Original Article

Absolute Lymphocyte Count; the most Sensitive Indicator of the Risk of Progression and Complications in COVID-19

Kamran Ali, Khurram Haq Nawaz*, Wahaj Ul Hassan, Raja Muhammad Waqar**

Department of Medicine, Pak Emirates Military Hospital/National University of Sciences (NUMS), Rawalpindi Pakistan, *Department of Medicine, Combined Military Hospital, Quetta/National University of Medical Sciences (NUMS), Pakistan, **Department of Medicine, Combined Military Hospital, Malir/ National University of Medical Sciences (NUMS), Pakistan

ABSTRACT

Objective: To explore the role of lymphocyte count as a predictor of severity biomarkers in COVID-19 patients.
Study Design: Comparative cross-sectional study.
Place and Duration of Study: Pak Emirates Military Hospital, Rawalpindi Pakistan, from Sep 2020 to Jan 2021.
Methodology: A total of 120 patients were enrolled for this study. We analyzed the blood samples of the affected patient in a certified laboratory. From blood samples, we deduced information related to leucocyte count, neutrophils, lymphocyte (cells×10^9/L) counts, ferritin (ng/mL), CRP (mg/dL), d-Dimer (mg/mL), lactate dehydrogenase (LDH, U/L) and lymphocyte subsets.
Results: In critical condition cases, we observed a massive decrease in CD3+CD4+T cell count by 49% (278+124.53 vs 545+138.32), CD4+/CD8+ratio by 30% (0.72+0.14vs1.00+0.04) and CD4+MFI by 17% (21834+1150.03 vs 26287+920.86) as compared to the Non-Critical Group (*p*-value<0.001). Comparatively, the ferritin and LDH levels were high in the Critical Group (*p*-value <0.001).
Conclusion: After the results, our study concluded that lymphocyte count plays a vital role in the early determination of COVID infection. We observed that a decrease in CD8+ count highly contributes to the severity of COVID-19.

Keywords: COVID-19, CD3+, CD4+ CD8+ count, Lymphocyte count.

How to Cite This Article: *Ali K, Nawaz KH, Hassan WU, Waqar RM. Absolute Lymphocyte Count; the most Sensitive Indicator of the Risk of Progression and Complications in COVID-19. Pak Armed Forces Med J 2023; 73(4): 1116-1119. DOI: https://doi.org/10.51253/pafmj.v73i4.6705*

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (https://creativecommons.org/licenses/by-nc/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

INTRODUCTION

With the widespread range of SARS-COVID-19 virus, many scientists claim that it will occasionally come in the future to target poor economic countries by causing a huge burden on the healthcare system.[1,2] Initial symptoms of this deadly virus were very similar to pneumonia, creating obstacles to distinguishing these two viruses at the initial stage.[3] Many researchers examined pulmonary opacity in COVID-19 patients with CT imaging.[4,5] Close contacts and respiratory droplets are the reason for the widespread of this virus. Some researchers claimed that the faeces of affected patients might transmit it.[6,7] In many regions, symptoms were mild to moderate, but some patients reported severe respiratory failure within 7-8 days of symptom onset.[8] Comorbidities and poor immune systems are vital in the high morbidity and mortality ratio. Up to 25% of severe cases need immediate ICU assistance.[4] In many critical patients, cytokine storms and immune system dysfunction were highly observable.[9] In recent years, very limited literature has been produced on the role of lymphocyte subsets in SARS-CoV-2 infection. One of the studies observed a decline in CD3+, CD4+, and CD8+ T cell counts and CD4+/CD8+ ratio in the early stage of SARS compared to other viral diseases like HIV or BVB infection.[6] Variations in lymphocyte count were observed at different phases of SARS infection to define its association with COVID-19, but still, the role of lymphocyte count needed to be studied.[10] This study was designed to explore the role of lymphocyte count as a predictor of severity biomarkers in COVID-19 patients.

Correspondence: Dr Kamran Ali, Department of Medicine, Pak Emirates Military Hospital, Rawalpindi Pakistan
Received: 06 May 2021; revision received: 02 Dec 2021; accepted: 08 Dec 2021

METHODOLOGY

The comparative cross-sectional study was conducted at Pak Emirates Military Hospital, Rawalpindi Pakistan, from September 2020 to January 2021. The study was conducted after ethical approval from the Hospital Administration and Research Committee (ERC number A/28/EC/281/2021).

Inclusion Criteria: Patients admitted with a respiratory rate ≥30 breaths per minute, PaO^2 <92% while on FiO^2 ≥0.35; PaO^2/FiO^2 ratio <200, need mechanical ventilation, were included.

Exclusion Criteria: Patients above 75 years, patients of chronic renal disorders; severe pulmonary obstructive

disease; history of chemotherapy and hemodialysis, were excluded.

The subjects admitted in COVID Intensive care and high dependency units were selected from an even number of beds at regular intervals every fortnight. Informed consent was taken from all patients at the start of the study. Information related to demographics, date of symptoms onset, admission date of patients, and active cardiovascular risk factors were collected from each patient. Information related to patient discharge and worst symptoms were also noted for this study. We analysed blood samples of affected patients in a certified laboratory. This information included observation of total leucocytes, neutrophils, lymphocyte (cells×10^9/L) counts, ferritin (ng/mL), CRP (mg/dL), d-Dimer (mg/mL), lactate dehydrogenase (LDH, U/L) and lymphocyte subsets. A total of 120 patients were enrolled for this study. Out of these 120, fifty patients were categorized as critically ill, and twenty-six underwent a thorough thoracic CT scan. Among these, ten patients had pulmonary thromboembolism along with COVID-19.

We analysed the lymphocyte subpopulation by adding a panel of monoclonal antibodies in blood samples. We used flow cytometry to collect the sample and analyzed it using the BD FACSuite™ Clinical Software version. Absolute numbers of cells were obtained using TruCount tubes. On the other hand, FlowJo software was used to measure the median fluorescence intensity (MFI) of different markers.

Statistical Package for Social Sciences (SPSS) version 23.0 was used for the data analysis. Quantitative variables were expressed as Mean±SD and qualitative variables were expressed as frequency and percentages. Independent sample t-test was applied to explore the inferential statistics. The *p*-value lower than or up to 0.05 was considered as significant.

RESULTS

In the limited timeframe of the study, we observed 50(41.6%) severe cases out of 120 recruited cases. The mean age of the patients was reported as 60.43+4.43 years, with mean days of admission for critical and non-critical patients being 15.46+3.28 and 4.94+0.81 days. Comorbidities like cardiovascular disorders were low, with 24(20%) hypertensives and 20(16.7%) dyslipidemics. During the follow-up, only 20(38.46%) patients under the category of critical illness expired.

In critical condition cases, we observed a massive decrease of 49%(278 vs 545) in CD3+CD4+T cell count (*p*-value<0.001),28%(0.72 vs 1.0) in CD4+/CD8+ratio (*p*-value<0.001) and 17%(21834 vs 26287) in CD4+ MFI(*p*-value<0.001) as compared to the Non-Critical Group. The absolute lymphocyte count was also lower in critical patients than in non-critical patients (*p*-value=0.001). There was a significant association between mortality outcome and low absolute lymphocyte count (*p*-value=0.001) (Table).

Table: Blood parameters of Critical and Non-Critical Patients (n=120)

Characteristics	Critically ill Patients n=52, ±SD	Non Critical Patientss n=68, ±SD	*p*-value
Leucocyte count (cells×10^9/L)	5970±1620	6550±1721	<0.001
Lymphocyte count (cells×10^9/L)	1116±290.5	1260±119.8	<0.001
Neutrophil count (cells×10^9/L)	4200±1579.2	4570±1315.6	<0.001
Neutrophil to Lymphocyte Ratio	4.0±4.0	3.57±1.1	<0.001
CD8±MFI	25337±3942.9	25916±840.9	<0.001
Ferritin (ng/mL)	783.7±498.4	639.1±200.4	<0.001
CD4±MFI	21834±1150.0	26287±920.9	0.001
CRP (mg/dL)	9.50±2.0	8.54±1.7	<0.001
Ratio CD4±/CD8±	0.72±0.13	1.0±0.04	<0.001
D-Dimer (mg/mL)	679±338.4	703±115.6	<0.001
Natural Killer count	234±54.0	192±37.2	<0.001
LDH (U/L)	356±59.6	267±33.1	<0.001
B Lymphocyte count	79±36.4	121±24.6	<0.001
T lymphocyte count	647±191.8	725±138.8	<0.001
CD3±CD4±count	278±124.5	545±92.2	<0.001
CD3±CD8±count	237±195.0	253±194.8	<0.001

DISCUSSION

This study showed a significant reduction in CD3+CD4+ T cell count, the CD4+/CD8+ ratio, and the CD4+MFI among patients at admission. Evaluation of these elements helps us to evaluate COVID-19 patients with bilateral pneumonia. Results of our study suggest that the investigation of CD3+CD4+ T cell count and expression level of CD4+ helps to decide the aggressive treatment with corticosteroids or IL-6 inhibitors at the initial stage of COVID-19 detection. In this study, we observed that the prediction of lymphocyte subsets assists us in treating critical patients with IL-6 inhibitors.

One previous study explained that late production of adaptive response and delay in the process of virus clearance had many contributions to severe COVID-19 development.[11] Sun *et al.* and Wang *et al.* observed a decline in CD3+, CD4+, and CD8+ T cell count in critical cases infected with the virus.[12,13] Though all those studies were conducted at the last

stage of disease (at the point of lung failure), they failed to evaluate the role of the lymphocyte subset at an early stage of disease as a prognostic factor of disease severity. Our results align with the previous animal model studies on SARS-CoV by Zao *et al.* in 2010. They observed that T lymphocytes are responsible for virus clearance.[14] A similar study done by Chen *et al*. in 2010 on SARS-CoV concluded that depletion of CD4+ T cells declined the neutralization process of antibodies. In contrast, depletion of CD8+ T cells was ineffective for neutralizing antibodies.[15] Their studies end with the assumption that CD4+ cell response is critical to control SARS-COVID-19 infection.

In this study, we categorized hospital-admitted cases of COVID-19 in severe cases and found an inverse association of CD3+CD8+ T cells and CD4+ MFI with the duration of hospital stay. We observed an association of CD3+CD4+ T cells with critically ill patients. In 2020, Jiang *et al.* observed a massive decline in CD8+ T cells among severe cases of COVID-19, which eventually caused the need for ICU admission.[16] Our results are in accordance with the previous studies,[17] which observed variations in CD3 and CD8+ T cell levels. These variations cause a delay in hospital discharge, which was identified in another study done by Sun *et al*.[18] Another retrospective study by Zheng *et al*. 2020 observed that decreased CD4+ and CD8+ T cell levels in critical patients cause malfunctioning in the immune system.[19]. Another retrospective study by Hengeveld *et al*. reported that COVID-19 damages the CD4+ T cells, which boosts the excessive activation of CD8+ T cells with potential exhaustion.[20] Our results are in accordance with the previously conducted retrospective study of Hengeveld *et al.* on hospitalized COVID-19 patients. We observed a massive decline in CD8+ and CD4+ cell count and reconstitution of CD8+ T cells at a later stage of the disease.

Many other viral diseases like HIV and herpes virus infection also observed a low level of CD4+ T cells during diagnosis due to the lipid raft's internalisation. In many SARS COVID-19 study by Hoffman *et al.* in 2021, a hypothesis emerged to support this evidence that COVID-19 cause damage to T cells through S protein-mediated membrane fusion.[21] Our study contributes to the pathophysiological understanding of viruses. We observed the CD3+CD4+ T cells involvement at the initial stage of the disease and observed excessive activation of CD8+ T cells at the chronic stage. These results align with the previous study of Ganji *et al*. in 2020, who observed high cytotoxic activity due to overexpression of CD8+ protein.[22]

CONCLUSION

After the results, our study concluded that lymphocyte count plays a vital role in the early determination of COVID-19 infection severity. We concluded our study with the results which define the specific role of CD4+ lymphocytes at the initial stage of diagnosis. We observed that CD8+ count highly contributes to the severity of COVID-19 infection.

Conflict of Interest: None.

Author's Contribution:

Following authors have made substantial contributions to the manuscript as under:

KA & KHN: Data acquisition, data analysis, critical review, approval of the final version to be published.

WUH & RMW: Study design, drafting the manuscript, data interpretation, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

REFERENCES

1. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet 2020; 395(10223): 497-506. https://doi.org/10.1016/s0140-6736(20)30183-5
2. Jin Y, Yang H, Ji W, Wu W, Chen S, Zhang W, et al. Virology, epidemiology, pathogenesis, and control of COVID-19. Viruses. 2020; 12(4): 372. https://doi.org/10.3390/v12040372
3. Giamarellos-Bourboulis EJ, Netea MG, Rovina N, Akinosoglou K, Antoniadou A, Antonakos N, et al. Complex immune dysregulation in COVID-19 patients with severe respiratory failure. Cell Host Microbe 2020; 27(6): 992-1000. https://doi.org/10.1016/j.chom.2020.04.009
4. Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J, et al. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus–infected pneumonia in Wuhan, China. JAMA 2020; 323(11): 1061-1069. https://doi.org/10.1001%2Fjama.2020.1585
5. Tay MZ, Poh CM, Rénia L, MacAry PA, Ng LF. The trinity of COVID-19: immunity, inflammation and intervention. Nat. Rev. Immunol 2020; 20(6): 363-374. https://doi.org/10.1038/s41577-020-0311-8
6. Janice Oh HL, Ken-En Gan S, Bertoletti A, Tan YJ. Understanding the T cell immune response in SARS coronavirus infection. Emerg Microbes Infect 2012; 1(1): 1-6. https://doi.org/10.1038/emi.2012.26
7. Calvet J, Gratacós J, Amengual MJ, Llop M, Navarro M. CD4 and CD8 lymphocyte counts as surrogate early markers for progression in SARS-CoV-2 pneumonia: a prosp-ective study. Viruses 2020; 12(11): 1277. https://doi.org/10.3390 /v12111277
8. COVID-19 coronavirus pandemic: Update. [Internet] available at: https://www.worldometers.info/coronavirus/. (Access on date September 6, 2020)
9. Wen W, Su W, Tang H, Le W, Zhang X, Zheng Y, et al. Immune cell profiling of COVID-19 patients in the recovery stage by single-cell sequencing. Cell Discov 2020; 6(1): 1-8. https://doi.org/10.1038/s41421-020-0168-9

10. Whitmire JK. Induction and function of virus-specific CD4+ T cell responses. Virol J 2011; 411(2): 216-228. https://doi.org /10.1016%2Fj.virol.2010.12.015
11. Channappanavar R, Zhao J, Perlman S. T cell-mediated immune response to respiratory coronaviruses. Immunol. Res 2014; 59(1): 118-128. https://doi.org/10.1007/s12026-014-8534-z
12. Sun DW, Zhang D, Tian RH, Li Y. The underlying changes and predicting role of peripheral blood inflammatory cells in severe COVID-19 patients: A sentinel? Clinica Chimica Acta 2020; 508: 122-129. https://doi.org/10.1016 %2Fj.cca.2020.05.027
13. Wang F, Nie J, Wang H, Zhao Q, Xiong Y, Deng L, et al. Characteristics of peripheral lymphocyte subset alteration in COVID-19 pneumonia. J Infect Dis 2020; 221(11): 1762-1769. https://doi.org/10.1093/infdis/jiaa150
14. Zhao J, Perlman S. T cell responses are required for protection from clinical disease and for virus clearance in severe acute respiratory syndrome coronavirus-infected mice. J Virol 2010; 84(18): 9318-9325. https://doi.org/10.1128/jvi.01049-10
15. Chen J, Lau YF, Lamirande EW, Paddock CD, Bartlett JH, Zaki SR, et al. Cellular immune responses to severe acute respiratory syndrome coronavirus (SARS-CoV) infection in senescent BALB/c mice: CD4+ T cells are important in control of SARS-CoV infection. J Virol 2010; 84(3): 1289-1301. https://doi.org/ 10.1128/jvi.01281-09
16. Jiang M, Guo Y, Luo Q. T-cell subset counts in peri-pheral blood can be used as discriminatory biomarkers for diagnosis and severity prediction of coronavirus disease 2019. J Infect Dis 2020; 222(2): 198-202. https://doi.org/ 10.1093/infdis/ jiaa252
17. Urra JM, Cabrera CM, Porras L, Ródenas I. Selective CD8 cell reduction by SARS-CoV-2 is associated with a worse prognosis and systemic inflammation in COVID-19 patients. Clin Immunol 2020; 217(1): 108486. https://doi.org/10.1016%2Fj.clim.2020.108 48665464564
18. Sun Y, Dong Y, Wang L, Xie H, Li B, Chang C, et al. Characteristics and prognostic factors of disease severity in patients with COVID-19: The Beijing experience. J Autoimmun 2020; 112(1): 102473. https://doi.org/10.1016/j.jaut.2020.102473
19. Zheng HY, Zhang M, Yang CX, Zhang N, Wang XC, Yang XP, et al. Elevated exhaustion levels and reduced functional diversity of T cells in peripheral blood may predict severe progression in COVID-19 patients. Cell. Mol. Immunol 2020; 17(5): 541-543. https://doi.org/10.1038/s41423-020-0401-3
20. Hengeveld PJ, Khader AO, de Bruin LH, Geelen IG, van Baalen EA, Jansen E, et al. Blood cell counts and lymphocyte subsets of patients admitted during the COVID-19 pandemic: a prospective cohort study. Br J Haematol 2020. https://doi.org/10.1111 /bjh.16983
21. Hoffmann HH, Sánchez-Rivera FJ, Schneider WM, Luna JM, Soto-Feliciano YM, Ashbrook AW, et al. Functional interrogation of a SARS-CoV-2 host protein interactome identifies unique and shared coronavirus host factors. Cell Host Microbe 2021; 29(2): 267-280. https://doi.org/10.1016/j.chom.2020.12.009
22. Ganji A, Farahani I, Khansarinejad B, Ghazavi A, Mosayebi G. Increased expression of CD8 marker on T-cells in COVID-19 patients. Blood Cells Mol Dis 2020; 83(1): 102437. https:// doi.org/10.1016%2Fj.bcmd.2020.102437