ORIGINAL ARTICLE

Association of Mortality with Lymphocyte Subset in Patients with COVID-19-associated Acute Respiratory Failure: A Subgroup Analysis

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Received on: 16 December 2022; *Accepted on:* 19 December 2022; *Published on:* 31 December 2022

Abstract

Background: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causes pneumonia and lymphopenia. We investigated the predictive value of T-lymphocyte subset absolute counts for outcomes following coronavirus disease-2019 (COVID-19)-associated acute respiratory failure (C-ARF).

Patients and methods: A retrospective chart review of adult patients with C-ARF was undertaken from 23 March 2020 to 20 November 2021 to obtain relevant data. Patients were divided into two groups based on survival. The T-lymphocyte subsets were determined by flow cytometric analysis. A binomial logistic regression was performed to ascertain factors affecting survival. Cut-off values to differentiate between survivors and non-survivors were identified with the receiver operating characteristic (ROC) analysis.

Results: A total of 379 patients were analyzed. Age was negatively correlated with survival. Non-survivors had significantly lower T-lymphocyte subset absolute counts than survivors. Serum ferritin levels were significantly higher in non-survivors. Baseline lymphocyte (%) and a subset were predictive of survival in patients [lymphocyte (%) <5.65%, CD3+ <321 cells/µL, CD4+ <205 cells/µL, CD8+ <103 cells/µL].

Conclusions: Lower T-lymphocyte subsets were associated with higher mortality in patients with C-ARF. Monitoring trends may help in identifying patients at increased risk of poor outcomes.

Keywords: Acute respiratory failure, COVID-19 infection, Outcomes, Severe acute respiratory syndrome coronavirus 2, T-lymphocyte subsets.

Indian Journal of Critical Care Medicine (2023): 10.5005/jp-journals-10071-24392

Highlights

- We identified cut-off values of T-lymphocyte subsets that were predictive of in-hospital mortality.
- Assessment of baseline T-lymphocyte subset counts guides in recognizing patients at risk for unfavorable outcomes.

Introduction

Severe acute respiratory syndrome coronavirus 2 led to the COVID-19 pandemic with a gamut of clinical manifestations varying from asymptomatic to acute respiratory failure ranging from mild-to-critical disease. Studies have explored demographic, clinical, and laboratory parameters associated with greater COVID-19 severity. Age, gender, comorbidities and lymphopenia influence the progression of COVID-19 toward a severe course.[1] Changes in T-lymphocyte subsets have been noted following infection with RNA viruses. Currently, the pathophysiology of COVID-19 is unclear.[2] Characteristics of the immune system and its role in clinical features and disease progression following COVID-19 infection are unfurling. Lymphopenia has been reported in patients with C-ARF caused by SARS-CoV-2.[3,4] We have reported a decrease in T-lymphocytes/subsets, especially in those aged more than 60 years, male gender, and with diabetes mellitus.[3] Diabetes mellitus as a comorbidity enhances dysregulated immune response and worsens prognosis.[5] A significant negative correlation was found between inflammatory biomarker, serum ferritin, and T-lymphocytes/subsets reflecting the degree of immune dysregulation.[3,6,7] Innate immune system is the first line of defense against the virus. Also, T-lymphocytes/subsets at baseline and trends have predicted in-hospital mortality.[8,9] Functional exhaustion of T-lymphocytes in addition to depletion also influences recovery.[10]

We aimed to understand the clinical utility of the T-lymphocyte subset to prognosticate outcomes in patients with COVID-19 and identified cut-off values for T-lymphocyte subsets and their correlation with the risk of in-hospital mortality.

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How to cite this article: Vadi S, Pednekar A, Suthar D, Sanwalka N, Rabade N, Ghodke K. Association of Mortality with Lymphocyte Subset in Patients with COVID-19-associated Acute Respiratory Failure: A Subgroup Analysis. Indian J Crit Care Med 2023;27(1):52–56.

Source of support: Nil

Conflict of interest: None

Patients and Methods

Study Design and Population

This is a subgroup analysis of a previously reported single-center retrospective study involving patients from 23 March 2020 to 20 November 2021 was conducted at a tertiary care center in Mumbai.[3] The study was approved by Institutional Ethics Committee (047/2021) and written, informed consent was waived.

Study Objectives

The endpoint of the study was to demonstrate whether T-lymphocyte subsets are prognostic markers for outcomes of patients with C-ARF. The effects of concomitant COVID-19 and diabetes mellitus on T-lymphocyte subsets and their relationship with prognosis were also examined.

Data Collection

We collected information on age, gender, history of diabetes mellitus, laboratory parameters [white blood cell count (WBC); T-lymphocyte subsets, CD3+, CD4+, CD8+; absolute lymphocyte count; and worst serum ferritin], maximum oxygen required, and outcomes. Blood sampling was done following COVID-19 intensive care unit (ICU) admission. These included direct admissions, and patients transferred from the COVID-19 ward or transferred to the hospital for further management. All blood tests were performed in the hospital's central laboratory adhering to the standard procedures. Laboratory data were cross-checked by two hematologists (KG and NR). Clinical data were cross-checked by three physicians (AP, DS, and SV).

Patient Classification and Definitions

Details of patient classification and definitions used in this T-lymphocyte/subset study have been published previously.[3]

Flow Cytometry

EDTA anticoagulated whole blood samples were used for T-cell subset enumeration. Briefly, 100 µL of the sample was stained with 10 µL of antibody cocktail (CD45-FITC/CD4-RD1/CD8-ECD/CD3-PC5) and processed using the standard stain-lyse-wash protocol.[3] A single platform method using fluorescent beads was used to enumerate the T-cell subset absolute counts. A total leucocyte count and the differential count were performed on a Sysmex XN1500 CBC analyzer, prior to processing to ensure that the total lymphocyte count was within the linearity range and as an additional quality check the total lymphocyte counts were compared with those obtained from the flow cytometry method. Processed samples were acquired using the 10-color Navios EX Flow cytometer (Beckman Coulter) and results were analyzed using automated gating and calculations on the Kaluza analysis software. The absolute number (cell/µL) of positive cells in the sample was determined by comparing cellular events to bead counts.

Normal ranges of T-lymphocyte subsets in healthy adults have been adopted from our previous study[3] and are as depicted in Table 1.

Table 1: Normal ranges of T-lymphocyte subsets in healthy adults

Cell type	*Unit*	*Biological reference range/interval*
WBC count	10^3/µL	4–11
CD3+ (T-lymphocyte) absolute count	Per µL	537–2,939
CD4+ (T-helper cells) absolute count	Per µL	321–2,124
CD8+ (T-cytotoxic cells) absolute count	Per µL	46–1,346

Statistical Analysis

The previous study[3] focused on analyzing T-lymphocyte subgroup when data were classified according to gender, the severity of COVID-19, the prevalence of diabetes mellitus, and the relationship of T-lymphocytes with blood levels whereas this study focuses on analysis based on mortality (survivors vs non-survivors).

Statistical package for the social sciences (SPSS) software, version 25, for Windows (version 25, 2017, IBM Corporation, Armonk, New York, USA) was used to perform analysis. Differences in parameters were analyzed using Mann–Whitney test. Categorical variables were cross-tabulated and compared using Fisher's exact test. Binomial logistic regression was used to analyze factors affecting the likelihood of survival in participants and presented as an odds ratio with a 95% confidence interval (CI). Receiver operating characteristic analysis with Youden's index was used to identify cut-off values to determine survival. Sensitivity, specificity, and area under the curve (AUC) were calculated. A $p < 0.05$ was considered to be statistically significant. Data presented as median [interquartile range (IQR)] or frequency (%).

The study followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting guideline.

Results

Flowchart of patients analyzed (Flowchart 1) depicts the CONSORT flow diagram of the patients analyzed.

Basic Demographics of Study Participants based on Outcomes

Table 2 presents the demographics of study participants. A significantly higher percentage of non-survivors had severe COVID-19 as compared to survivors ($p < 0.05$). A higher percentage of patients had diabetes mellitus in the survivor group as compared to the non-survivor group ($p < 0.05$). Survivors were significantly younger as compared to non-survivors ($p < 0.05$).

The T-lymphocyte and Subset Counts to Outcomes

Figure 1 give T-cell counts and WBC when classified according to the outcome. Non-survivor patients had significantly lesser total lymphocyte (%), cd3+ (absolute), cd4+ (absolute), and cd8+ (absolute) count as compared to survivors ($p < 0.05$). Survivors had significantly lower WBC levels as compared to non-survivors ($p < 0.05$).

The HbA1c and Serum Ferritin Levels according to Outcomes

Figure 2 give HbA1c and serum ferritin levels when classified according to the outcome. Survivors had significantly lower serum ferritin levels as compared to non-survivors ($p < 0.05$). On the other hand, HbA1c was significantly higher in survivors as compared to non-survivors ($p < 0.05$).

Flowchart 1: Flowchart of patients analyzed

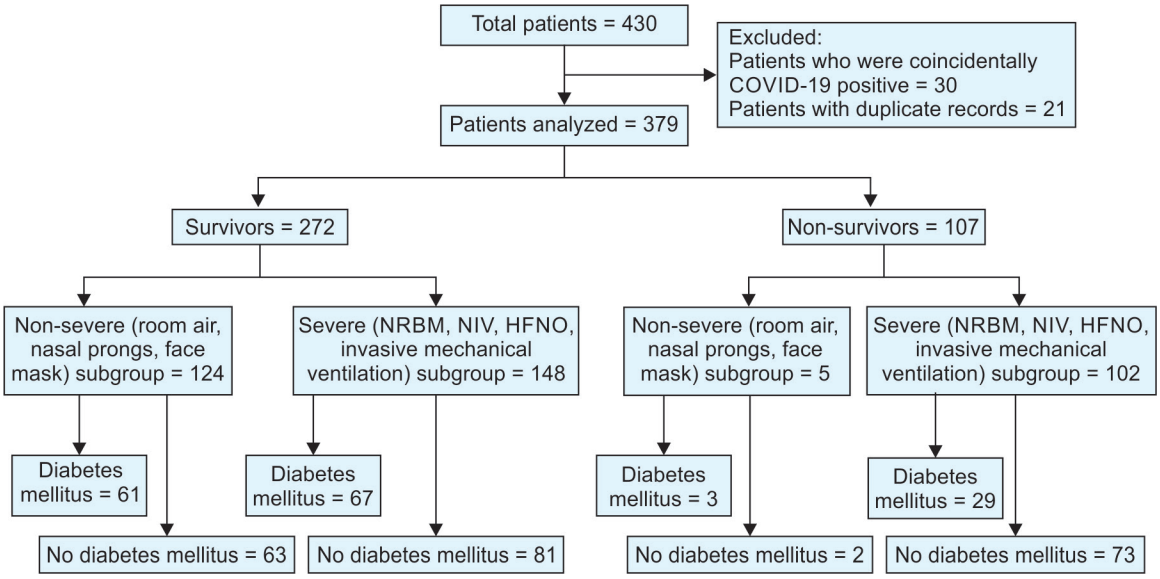


Table 2: Basic demographic of study participants

	Survivors (n = 272)	*Non-survivor (n = 107)*	*Total (n = 379)*	*p-value*
Age*				
Age (years)	61 (18)	67 (21)	62 (18)	0.002
Gender**				
Male	189 (69.5%)	77 (72%)	266 (70.2%)	0.709
Female	83 (30.5%)	30 (28%)	113 (29.8%)	
Diabetes mellitus**				
Yes	128 (47.1%)	32 (29.9%)	160 (42.2%)	0.003
No	144 (52.9%)	75 (70.1%)	219 (57.8%)	
Severity of illness**				
Non-severe	124 (45.6%)	5 (4.7%)	129 (34%)	0.001
Severe	148 (54.4%)	102 (95.3%)	250 (68%)	

*Data presented as median (IQR); **Data presented as frequency (percentage)

Logistic Regression to Determine Factors affecting Outcome

A binomial logistic regression was performed to ascertain the effects of severity of illness, presence of diabetes mellitus, age, lymphocyte (%), CD4+ absolute count, and serum ferritin levels on the likelihood of survival (Table 3). The overall percentage accuracy in classification was 79.2%, that is, the model correctly identified the outcome in 79% of the cases. Sensitivity was 88.2% (95% CI: 83.4–91.9), specificity was 55.4% (95% CI: 44.7–65.8), positive predictive value was 84.05% (95% CI: 80.7–86.9), and negative predictive value was 63.8% (95% CI: 54.4–72.2). Of the six predictor variables, the following four were statistically significant: Age, the severity of illness, lymphocyte (%), and CD4+ absolute count. The odds of being a non-survivor were 10.5 times greater for patients with severe COVID-19 as compared to patients who had non-severe COVID. Increasing age was associated with an increased likelihood of not surviving, and increasing lymphocyte (%) and CD4+ absolute count was associated with an increased likelihood of surviving.

The ROC Analysis

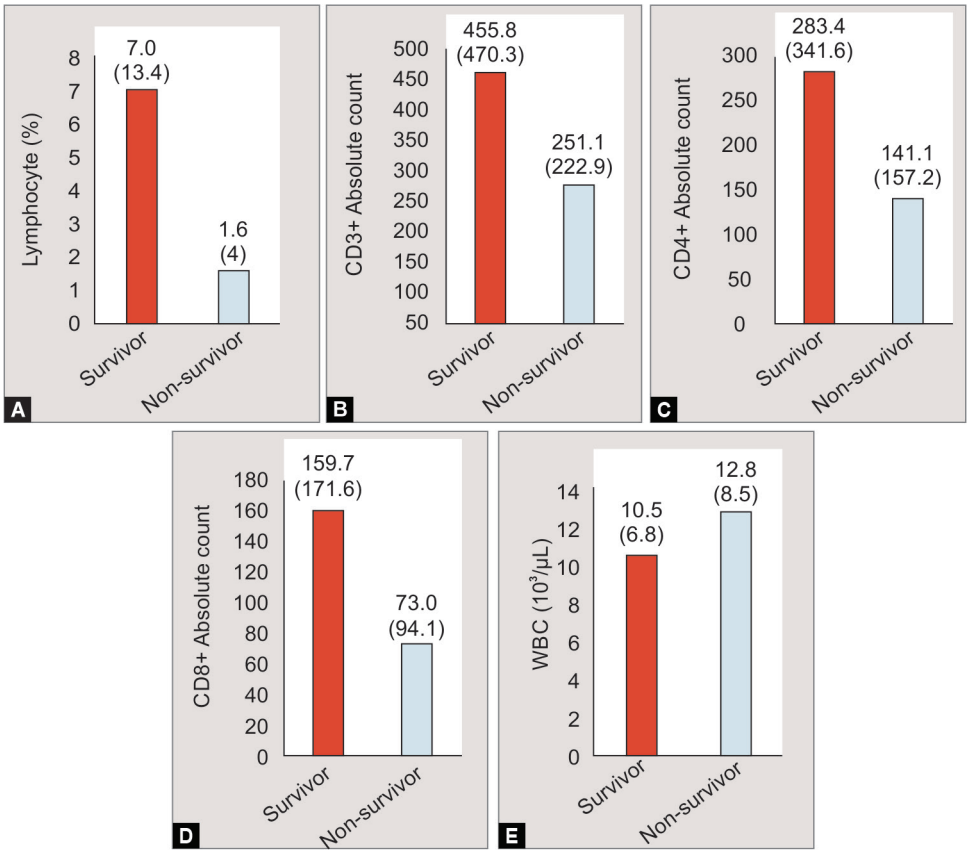
The ROC analysis showing the performance of baseline absolute counts of total T-lymphocytes on survival is presented in (Fig. 3). Cut values for identifying non-survivors from survivors were determined using Youden's index. The cut-off values were as follows: Lymphocyte (%) <5.65% (sensitivity 56.5%, specificity: 84.8%, AUC – 0.745); CD3+ <321 cells/μL (sensitivity, 73.8%; specificity, 71.4%; AUC, 0.776); CD4+ <205 cells/μL (sensitivity, 69%; specificity, 72.4%; AUC, 0.758); CD8+ <103 cells/μL (sensitivity, 73.8%; specificity, 68.6%; AUC, 0.795) ($p < 0.001$).

Discussion

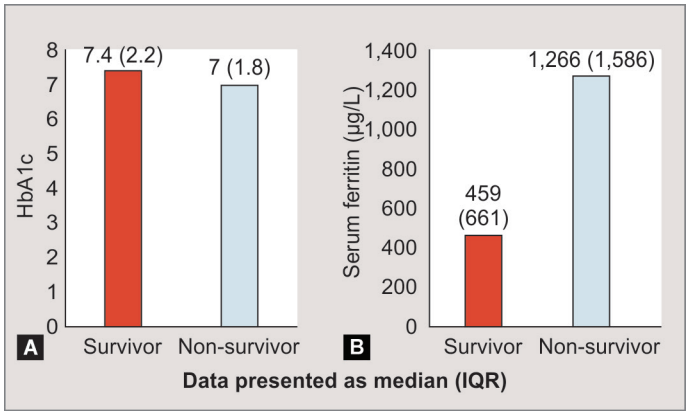
We retrospectively analyzed laboratory and clinical parameters of 379 patients hospitalized with SARS-CoV-2 infection and elucidated the ability of T-lymphocyte subset absolute counts to predict outcomes in patients with C-ARF. Studies[5,6] have shown age and male gender as independent factors associated with unfavorable outcomes. Our study confirmed these results. Increasing age was associated with a likelihood of not surviving. Higher CD4 absolute count was associated with survival. Lymphopenia is associated with the severity of the disease and can be used as a predictive marker of hospital mortality. Release of pro-inflammatory chemokines and cytokines leads to the "cytokine storm" in COVID-19 infection. In our cohort, serum ferritin levels were increased and correlated negatively with survival.

Additionally, ROC analysis gave us cut-off values for T-lymphocyte/subsets predictive for in-hospital mortality. Clinically, these cut-off values can be used as prognostic indicators in patients with C-ARF.

Being retrospective in nature, our study could be associated with a systemic selection bias. A prospective validation cohort study



Figs 1A to E: The T-cell counts and WBC when classified according to the outcome



Figs 2A and B: The HbA1c and serum ferritin levels when classified according to the outcome

will help strengthen the findings of our study. In this cross-sectional study, we analyzed T-lymphocytes/subsets at a critical phase of illness when patients were transferred to COVID-19 ICU. Monitoring T-lymphocyte/subset trends may provide quicker information on dynamics of severity to probably implement optional treatments once available from future research.

Studies have described T-lymphocytes/subsets to assess the risk of mortality in COVID-19 patients.[4,11] Results have been consistent[11] despite varying definitions of disease severity, clinical care provided, and blood sample acquisition timings. Our single-center study from Mumbai, India accords with the published data.

Considering variabilities in treatment considerations in different regions these results warrant further validation to generalize findings to patients of different regions. Social determinants of health and risk factors vary amongst populations. Further studies on prognostic value for outcomes in differing COVID-19 cohorts including obstetric population are needed.

Table 3: Logistic binomial regression to determine factors affecting the outcome

				95% CI for Exp (B)	
	B	*p-value*	*Exp (B)*	*Lower*	*Upper*
Age	0.039	0.001	1.039	1.015	1.064
Presence of diabetes mellitus	−0.314	0.313	0.730	0.396	1.345
Severity of COVID-19	2.353	0.000	10.513	3.496	31.614
Lymphocyte (%)	−0.097	0.005	0.908	0.848	0.972
CD4+ absolute count	−0.004	0.001	0.996	0.994	0.998
Serum ferritin	0.000	0.109	1.000	1.000	1.000

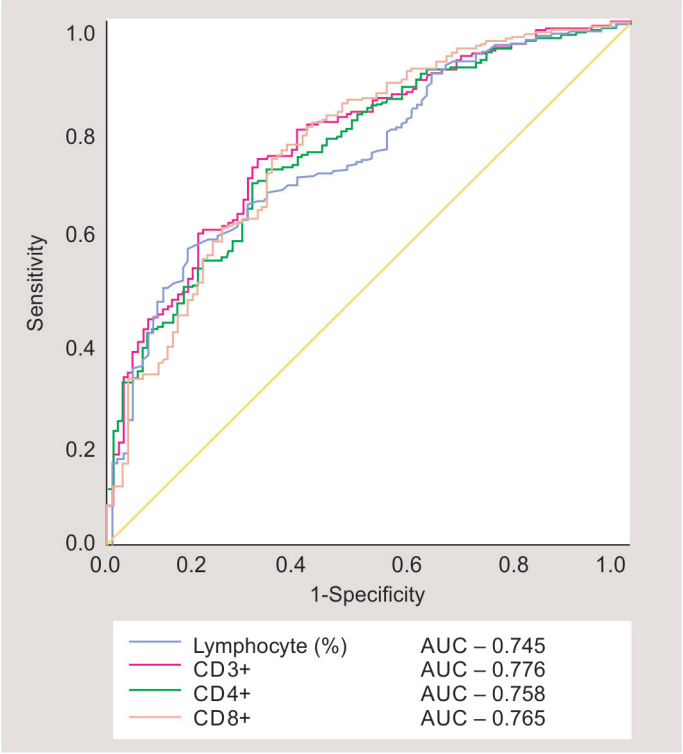


Fig. 3: The ROC analysis of T-lymphocyte subset absolute counts for survival rate. The ROCs are represented for T-lymphocyte cells and subsets

Conclusion

Findings from our study may help to further understand the pathogenesis of COVID-19. Assessment of T-lymphocyte subset absolute counts will help identify patients at increased risk for progression and adversarial consequences following C-ARF and customized therapeutic options for those with severe disease.

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