Microbes and Infectious Diseases 2022; 3(1): 24-35

Microbes and Infectious Diseases

Journal homepage: https://mid.journals.ekb.eg/

Original article

Evaluation of peripheral lymphocyte subsets' alteration and IL6 serum level correlated with severity and outcome in corona virus disease 2019 (COVID-19)

Manar Magdy Abd Elhady*[1], Amany T. Abd Elrahman [2], Nesma Gamal Ahmed Elsayed Elsheikh [3], Noha N. Salah El-Deen [2]

1- Medical Microbiology and Immunology Department, Faculty of Medicine, Damietta University, Egypt.
2- Medical Microbiology and Immunology Department, Faculty of Medicine, Ain Shams University, Egypt.
3- Geriatric and Gerontology Medicine Department, Faculty of medicine, Ain Shams University, Egypt.

ARTICLE INFO

Article history:
Received 20 September 2021
Received in revised form 21 October 2021
Accepted 28 October 2021

Keywords:
COVID19
IL6
Lymphocyte subsets
Severe infection

ABSTRACT

Background: Coronavirus disease 2019 (COVID-19) has rapid spread worldwide and its pathogenesis is still not well understood. It's critical to identify the key immune inflammatory markers that may be correlated with COVID-19 severity. **Objective:** This study aimed to study the association of the peripheral lymphocyte subsets' alteration and IL-6 serum level with disease severity and outcome in COVID-19. **Methodology:** Samples from 30 COVID-19 patients were collected; one is EDTA anticoagulated for flowcytometric analysis of different lymphocyte subsets and the other for Interleukin-6 (IL6) serum level assessed by ELISA technique. **Results:** Absolute lymphocytic count (0.9 (0.5 - 1.4)× 10^3/µL) , $CD4^+$ T cells (217 (135.6 - 445.5) cells/µL) , $CD8^+$ T cells (160 (112 - 338) cells/µL) and natural killer (NK) cells (33.3 (18.2 - 99.5) cells/µL) were significantly reduced in severe COVID-19 patients with significantly elevated IL-6 serum levels 90 (70-120) (pg/mL) in severe patients. Lower T lymphocytes and NK subset counts with higher IL-6 levels were significantly associated with higher mortality. However, B cell count was not associated with severity or mortality. Il-6 levels, $CD4^+$ and $CD8^+$ T cells counts were considered best predictors of disease severity and mortality according to ROC curve analysis (with AUC 0.842, 0.884 and 0.773 respectively). **Conclusion:** Peripheral lymphocyte subsets as $CD4^+$ T cells, $CD8^+$ T cells and NK cells were significantly reduced in severe COVID-19 patients. $CD4^+$ T cell count was the most significant biomarker for disease severity .Serum IL-6 levels were higher in severe illness. So, IL-6 can serve as a significant predictor of COVID-19 severity. As regard mortality and relation with lymphocytic count and lymphocytic subsets, total lymphocytic count and all T lymphocyte subsets $CD4^+$, $CD8^+$ and $CD56^+$ cells count can be used as a significant predictor of death in COVID-19 patients. However, $CD19^+$ cells counts had no relation with death.

Introduction

The outbreak of coronavirus disease 2019 (COVID-19) was caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), this new pathogen belongs to the beta-

DOI: 10.21608/MID.2021.97001.1194
* *Corresponding author:* Manar Magdy Abdelhady
E-mail address: Dr.manarmagdy92@gmail.com

coronaviruses. The outbreak began in December 2019 in Wuhan, China and continued to spread worldwide. By March 11, 2020, it reached 115 countries [1-3].

World Health Organization (WHO) considered COVID-19 as a pandemic. More than 209 million COVID-19 confirmed cases were reported, including more than four million deaths worldwide till August 2021, with mortality rate about 4.2% [4].

More than 285,000 confirmed cases had been reported in Egypt till August 2021 with more than 16,000 confirmed deaths. Egypt witnessed 3 COVID-19 waves till now; the peak of the first wave was in June 2020 and the second wave was in November and December 2020. In late December 2020 and early January 2021, the number of cases declined. However, infections started to increase again in March 2021 to start the third wave [5].

Coronavirus disease 2019 has an extremely variable course ranging from no or mild manifestations to serious illness [6]. It predominantly affects the respiratory system, resulting in manifestations like fever, sore throat, cough, dyspnea, as well as respiratory failure and fatalities. In addition, extrapulmonary manifestations had been reported including cardiovascular, gastrointestinal, neurological, or dermatologic affection [7].

Coronavirus disease 2019 severity is affected by several factors like advanced age, male gender and underlying health problems as diabetes, hypertension, obesity, heart diseases, tumors, chronic respiratory illness and others [8].

Pulmonary and extra-pulmonary manifestations of SARS-CoV-2 result from the widespread expressed angiotensin converting enzyme 2 receptors used by the virus to enter human cells, that are generously expressed in lung epithelial cells [9]. The immunopathological features of the disease could be shown in a state of hypercytokinemia called a cytokine storm was correlated with aggressive pulmonary damage in SARS, Middle East Coronavirus Respiratory Syndrome (MERS-CoV) infection and lately in COVID-19 [10]. Interleukin-6 (IL-6) is one of the primary cytokines implicated in the cytokine storm and affects the disease seriousness so IL-6 is suggested as a predictor of COVID-19 severity and respiratory failure [11,12].

In the immune response against viral infections, different lymphocyte subsets like $CD4^+$ T cells, $CD8^+$ T cells, B cells, and natural killer (NK) cells have a key role in the humoral and cytotoxic immunity. The change in total lymphocytic count and subsets differs with different viral infections, suggesting a likely link between lymphocyte subset alteration and pathogenicity mechanism of the virus [13]. In laboratory-confirmed COVID-19 patients, several clinical studies have revealed peripheral lymphocytopenia [14].

Aim of the study

To study the association between peripheral lymphocyte subsets alteration and IL-6 serum level with disease severity and outcome in COVID-19 in order to guide physician in the management plan.

Patients and Methods

Study design and participants

This prospective study was performed at Ain Shams University Isolation Hospitals, Cairo, Egypt during the period from July 2020 to September 2020. The study was approved by the ethical and moral committee of Faculty of Medicine Ain Shams University (No. FMASU M S 416/2020). Thirty (30) COVID-19 patients participated in this study were divided into 2 subgroups; the first subgroup included 15 moderate cases and the second group included 15 severe cases. Classification was performed according to the Ain Shams Guide for COVID-19 (The criteria for classifying patients according to disease severity are included in **appendix (1)**.

Diagnosis of COVID-19 patients was confirmed by quantitative RT-PCR (qRT-PCR) and radiologically by chest CT findings.

Sample size was calculated by the community department Ain Shams University as a part of the ethical committee using PASS11 program for sample size calculation and according to **Qin et al.** [10] sample size of 15 persons per group can detect differences between 2 groups with power >90% setting alpha error at 0.05

Exclusion criteria

- Patients with autoimmune diseases, cancers or end stage diseases.
- Patients receiving anti-IL6 antibody treatment.

Data collection

Data collection included demographic data, symptoms, signs, medical history and laboratory findings of the study participants.

Sample collection

Ten ml blood sample was taken from each patient, 5 ml was collected in EDTA containing tubes for flowcytometric analysis within 24 hours and the other 5 ml was allowed to clot for separation of

serum which was stored at -20 till used for IL-6 quantitation by ELISA.

Flowcytometric analysis quality control using isotope controls

Flowcytometric analysis was done for different lymphocyte subsets ($CD3^+CD4^+$, $CD3^+CD8^+$ T-cell, $CD19^+$ B-cell, and $CD3^-$ $CD56^+$ NK cells). Blood samples were mixed with 2ml lyse solution then lymphocyte subsets were obtained by Ficoll gradient centrifugation after 5 minutes incubation at room temperature. Human monoclonal anti-CD3 Phycoerythrin - cyanine (PC5), anti-CD8 Phycoerythrin (PE), anti-CD4 Fluorescein isothiocyanate (FITC), anti-CD56 PE and anti-CD19 PE antibodies (Beckman Coulter Co., USA) were added to the freshly collected PBMCs. Antibody-treated cells were then vortexed and incubated for 15 minutes at room temperature in the dark. Final wash and centrifugation was done then cells were resuspended in PBS. The percentage of lymphocyte subsets was measured using a NAVIOS flowcytometer (Navios software version 1.2., Beckman Coulter Co., USA). A representative dot plot, obtained from flowcytometric data acquisition and analysis is included in Appendix 2. A hemocytometer was used to count the total lymphocytic count in the peripheral blood. By multiplying the percentages with the total lymphocyte count, the absolute count of different subsets of lymphocytes was obtained.

Detection of human IL-6 by ELISA

Before receiving any treatment, peripheral blood samples (5ml) were drawn from all patients. After centrifugation, serum samples were separated and IL-6 levels were measured using human IL-6 ELISA kit bioassay technology lab, Cat. No: E0090Hu, China) as described in the manufacturer's instructions [15].

Statistical analysis

All statistical analyses were performed using Statistical package for Social Science (SPSS 25). Statistics used in the different analyses included Student t-test, Mann Whitney Test (U test), Chi-Square test, Fisher's exact test and The Kruskal-Wallis test.

Results

The current study was conducted on 30 COVID-19 cases. They were classified into two equal groups; the first was 15 moderate COVID-19 patients (5 females, 10 males), and the second was 15 severe COVID-19 patients (4 females, 11 males).

As regard patients' age, those with severe infection were significantly older compared to the moderate group. Both groups were nearly equal in number as regard gender.

Twenty four (24) patients involved in this study had co-morbidities, the most common was hypertension and diabetes followed by heart then chest diseases and less common was renal and neurological diseases. Severe cases had more co-morbidities than the moderate, but the difference was statistically not significant except for cardiac diseases (*p value* < 0.01) **(Table 1).**

As regard the clinical outcome, total deaths among both groups were 20%. No deaths among the moderate group with 100% recovery while 40% of the severe group died with significant difference between both groups as regard the outcome *(P-value = 0.017)* **(Figure 1)**.

Absolute lymphocytic count among the study participants was decreased among 80% of them, with lower absolute lymphocytic count seen among severe group [median 0.9×10^3 cells/uL] than that in moderate patients' group [median 1.4×10^3 cells/uL]. By comparing the lymphocyte subset counts among the studied population, $CD4^+$ and $CD8^+$ T cells and $CD56^+$ natural killer cells counts were reduced significantly in severe patients in comparison to moderate patients and it was statistically significant ($P \leq 0.001$), While $CD19^+$ B cell counts did not vary significantly between both groups as shown in **table (2)** and **figure (2).**

Comparing results of lymphocytic count in association with mortality revealed that patients who died had lower lymphocytic count than those who survived [median (IQR) 0.55 (0.2 - 0.9) $\times10^3$ cells/uL] and [median (IQR) 1.4 (1.25 - 1.75) $\times10^3$] respectively, so this link lymphocytic count with severity of diseases as well as the outcome **(Table 3).**

As regard relationship between different peripheral lymphocyte subsets of participants and the outcome, patients who died reported lower numbers of $CD3^+CD4^+$, $CD3^+CD8^+$, and $CD3^-$ $CD56^+$ cells than those who survived and it was statistically significant *($p \leq 0.01$)*, while there was no statistical association between mortality and the difference in $CD19^+$ cell count.

Serum IL-6 level was measured among COVID-19 patients enrolled in the study and showed that patients with severe COVID-19 had elevated IL-6 serum levels [median 90 pg/mL], while patients with moderate infection had lower IL-6 levels [median 50 pg/mL], suggesting that cytokine storms may be linked to COVID-19 severity **(Table 2)**. There was no significant relation between IL-6 levels and patients' outcome **(Table 3).**

Receiver operating characteristic curve (ROC) curve analysis was done for different lymphocyte subsets, $CD3^+CD4^+$, $CD3^+CD8^+$ T cells and $CD3^-CD56^+$ NK cells can be considered significant predictors of COVID-19 severity *(P-Value < 0.01)*, while $CD19^+$ B cells had no statistical significance as regard disease severity **(Figure 3).** $CD3^+CD4^+$ T cell count was the most significant predictor of disease severity among lymphocyte subsets markers as it had the highest AUC=0.884 followed by $CD3^+CD8^+$ T cells with AUC= 0.773 as shown in **figure (3).**

ROC curve analysis was performed for IL-6 serum level. At a cut-off point of >60 pg/ml, IL-6 can significantly differentiate severe from moderate COVID-19 patients and predict disease severity with high sensitivity and specificity *(p-value <0.001)* **(Figure 4)**.

Table 1. Demographic characteristics and co-morbidities among the studied COVID-19 patients.

		Groups		Test of significance		
		Moderate COVID-19 patients (N=15)	**Severe COVID-19 patients (N=15)**	Value	*p*-value	Sig.
Age (Mean ± SD)		50.87 ± 19.32	66.4 ± 12.3	***t***=-2.626	0.015(t)	S
Gender N (%)	Male	10 (66.67%)	11 (73.33%)		1.00(F)	NS
	Female	5 (33.33%)	4 (26.67%)			
Co-morbidity N (%)	Diabetes	8 (53.33%)	7 (46.67%)		1.00(F)	NS
	Hypertension	8 (53.33%)	12 (80%)	X^2=2.4	0.121(C)	NS
	Chest disease	0 (0%)	1 (6.67%)		1.00(F)	NS
	Neurological	2 (13.33%)	1 (6.67%)		1.00(F)	NS
	Renal disease	1 (6.67%)	2 (13.33%)		1.00(F)	NS
	Cardiac disease	4 (26.67%)	10 (66.67%)	X^2=4.821	0.028(C)	S

(F)Monte Carlo Fisher's Exact test of significance.
Chi-Square test of significance X2= Chi-Square test value (C)
COVID-19, coronavirus disease 2019

Table 2. Absolute lymphocytic count, IL6 serum level and lymphocyte cell markers measured in moderate and severe patients' groups.

	Groups		Mann-Whitney test		
	Moderate COVID-19 patients (N=15)	**Severe COVID-19 patients (N=15)**			
	Median (IQR)	Median (IQR)	***U***	*p*-value	Sig.
Lymphocytes absolute count ($\times10^3$/µL)	1.4 (1.3 - 2.13)	0.9 (0.5 - 1.4)	42.5	0.004	S
IL6(pg/mL)	50 (45 - 55)	90 (70 - 120)	35.5	0.001	S
$CD3^+CD4^+$T cell (cell/µL)	506.8 (472 - 645.6)	217 (135.6 - 445.5)	26.0	<0.001	S
$CD3^+CD8^+$ T cell (cell/µL)	317 (243 - 673.2)	160 (112 - 338)	51.0	0.011	S
$CD3^-CD56^+$NK cell (cell/µL)	95 (57.4 - 181)	33.3 (18.2 - 99.5)	55.5	0.018	S
$CD19^+$B cell (Cell/µL)	129 (15 - 291.6)	69.6 (25 - 114.4)	77.0	0.141	NS

COVID-19, coronavirus disease 2019

Table 3. Association of absolute lymphocytic count, IL-6 serum level and lymphocyte subset changes with outcome of COVID-19 patients.

		outcome		Test of significance		
		Recovered (N=24)	**Died (N=6)**	Value	*p*-value	Sig.
Lymphocytes absolute count (x10^3/µL)	Median (IQR)	1.4 (1.25 - 1.75)	0.55 (0.2 - 0.9)	53.0	0.325	S
IL-6 (pg/mL)	Mean ± SD	65.71 ± 29.79	94.5 ± 30.75	***U***=34.5	0.051$^{(M)}$	NS
	Median (IQR)	52.5 (49 - 82.5)	102.5 (70 - 120)			
CD3$^+$CD4$^+$ T cells (cells/µL)	Mean ± SD	553.39 ± 427.56	133.32 ± 95.89	***U***=8.0	0.001$^{(M)}$	S
	Median (IQR)	489.5 (355.5 - 586.25)	130.25 (35 - 217)			
CD3$^+$CD8$^+$ T cells (cells/µL)	Mean ± SD	387.99 ± 238.89	101.42 ± 67.69	***U***=9.0	0.001$^{(M)}$	S
	Median (IQR)	313.5 (208.5 - 509.5)	111.25 (43.4 - 141)			
CD3$^-$CD56$^+$ NK cells (cells/µL)	Mean ± SD	103.75 ± 79.01	39.9 ± 28.6	***U***=32.0	0.038$^{(M)}$	S
	Median (IQR)	94.4 (38 - 144.5)	32.45 (13 - 73)			
CD19$^+$ B cells (cells/µL)	Mean ± SD	145.33 ± 147.36	56.07 ± 42.43	***U***=41.5	0.108$^{(M)}$	NS
	Median (IQR)	56.07 ± 42.43	61.1 (13.2 - 83.7)			

$^{(M)}$Mann-Whitney test of significance (U= Mann-Whitney test value).

Figure 1. Outcome of COVID-19 patients according to disease severity.

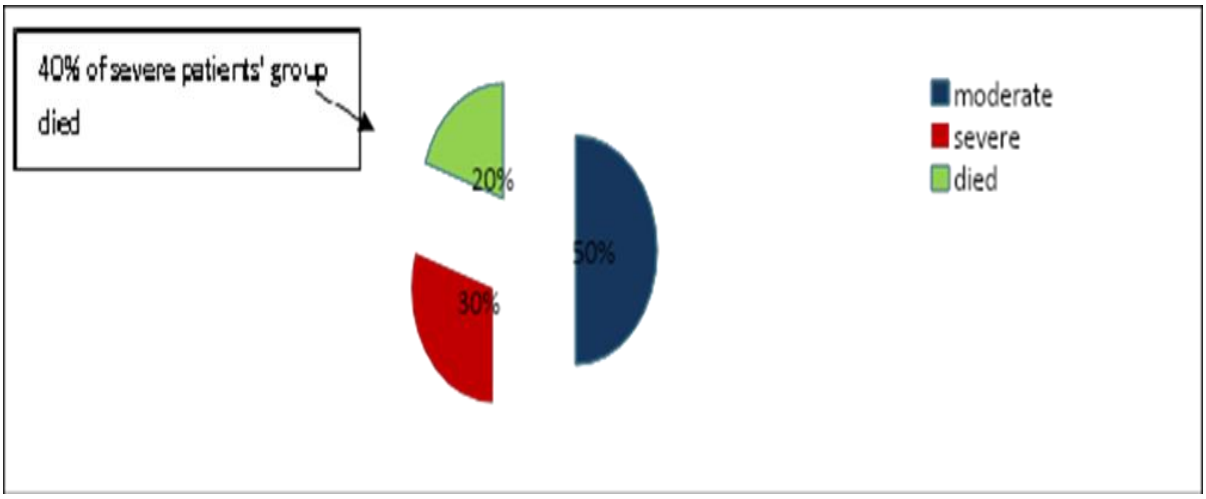


Figure 2. Comparison of median of different lymphocyte cells' count among COVID-19 patients according to disease severity (cells/uL).

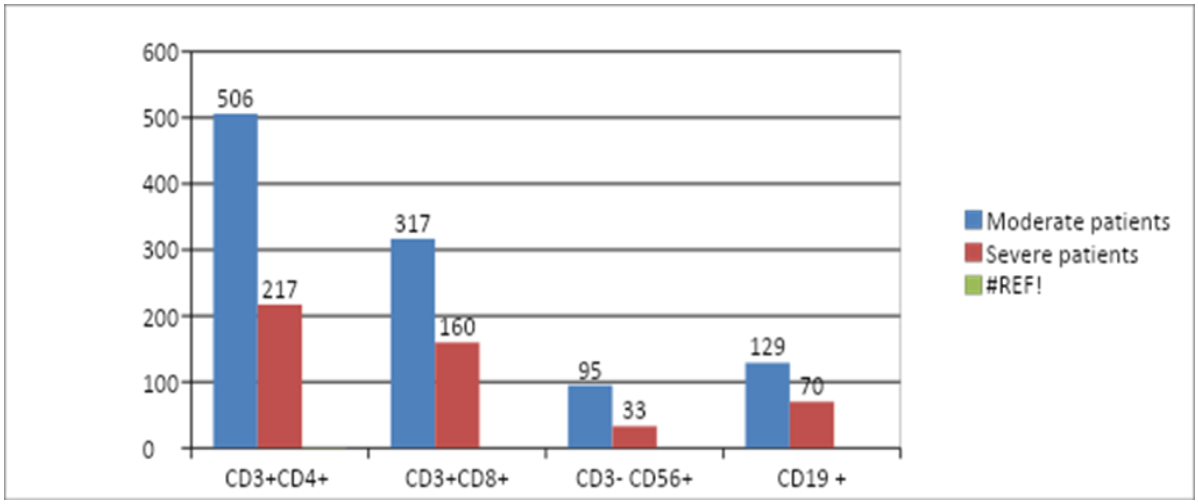


Figure 3. Comparison of ROC curves for different lymphocytic subsets in differentiation and prediction of COVID-19 severity among patients.

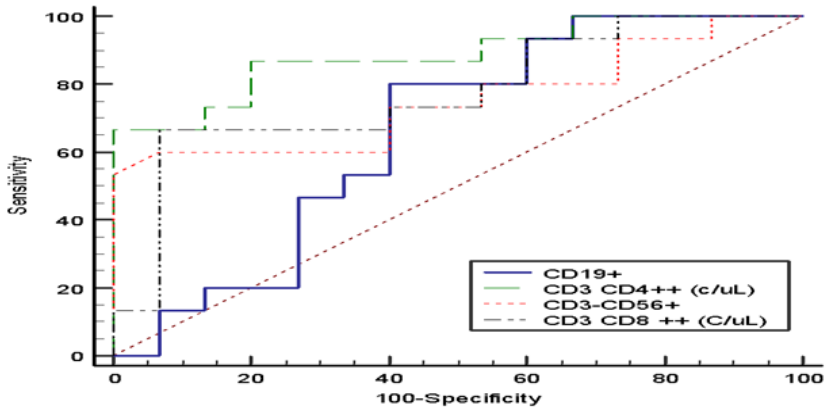
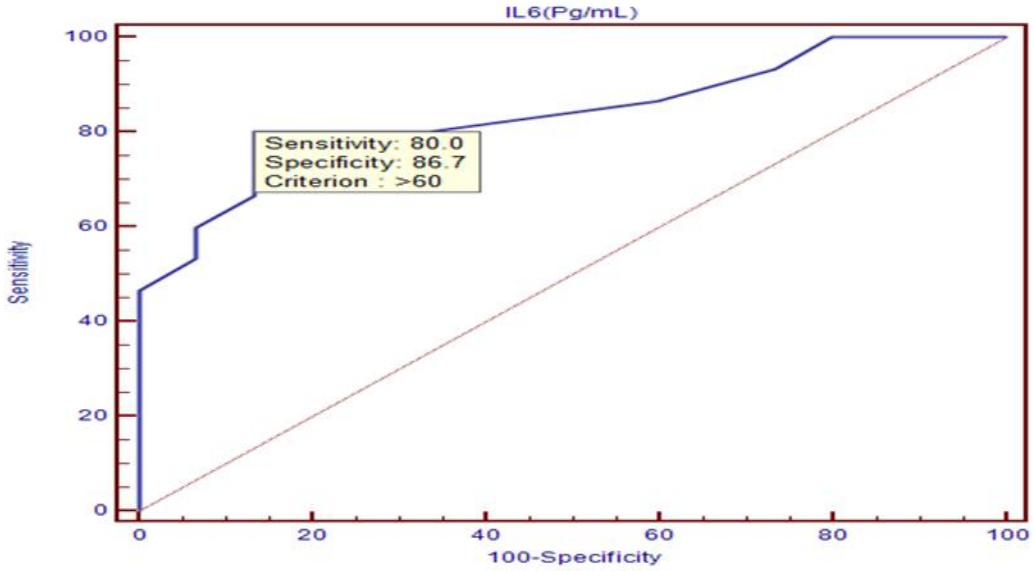


Figure 4. ROC curve analysis for IL-6 serum level (pg/mL) to predict severity of COVID-19 among the patients.



Discussion

Severe acute respiratory syndrome-CoV-2 became center of investigation since late 2019 due to the emergence of pandemic worldwide, resulting in variable symptoms and ranging in severity from mild to severe cases with high mortality rate [16]. Infection with SARS-CoV-2 can result in immune system dysregulation like other viral infections. Lymphocyte subsets have a significant role in maintaining the immune system regulation and fighting against different infections [10]. T-helper lymphocytes ($CD3^+CD4^+$) and T cytotoxic lymphocytes ($CD3^+CD8^+$), B lymphocytes ($CD19^+$), and NK cells ($CD3^-$ $CD56^+$) carry an important function in humoral and cytotoxic immunity. As a result, it's critical to spotlight the alteration in different lymphocyte subsets in COVID-19, which might provide new insights into the immunological response to COVID-19.

Results of this study linked age of the patients with severity of disease as older age were more prone to have a severe form of COVID-19 infection (mean 66 ± 12.3 years in severe group vs. 50.87 ± 19.32 years in moderate group; *P < .001)*. Among the severe group, about 73.33% were above 60 years old. There was male predominance among COVID-19 infected patients among both moderate and severe cases, suggesting males are more susceptible to SARS-CoV-2 infection than females. However, there was no statistical significant association with disease severity.

Qian et al. [17] agrees with the current study as they reported association between COVID-19 severity and old age.

Zhang and colleagues, [18] studied the relation between gender and COVID-19 severity and found non statistically significant difference. However, **Wang and colleagues,** [13] recorded female predominance among COVID-19 patients as 63% of the patients in their study were females.

The severe patients involved in the study had more co-morbidities than the moderate group. Hypertension and diabetes followed by heart diseases were the most common with non statistical significace except for cardiac diseases.

Qian et al. [17] and **Khamiss et al.** [19] also studied comorbidities among COVID-19 patients; hypertension and diabetes were the most frequent co-morbidity followed by COPD and cardiovascular diseases which was similar to the present study.

The current study revealed that most COVID-19 patients showed absolute lymphopenia (80%), which may be due to immune system dysfunction during SARS-CoV-2 infection which further worsen the clinical manifestations and increase rate of mortality. So lymphocytic count could be a predictor of mortality.

These results were compatible with **Qin et al.** [10] who assessed the absolute lymphocytic count among COVID-19 cases and found that severe cases had lower absolute lymphocytes count than non-severe cases (median 0.8 vs. 1.0×10^3 cells/μL)**.**

In the current study $CD3^+CD4^+$ and $CD3^+CD8^+$ T cells and $CD3^-CD56^+$ cells count was significantly reduced in severe patients compared to the moderate patients while B cell count didn't show significant change *(p-value <0.01)*.

These results were compatible with **Deng and colleagues,** [14] who stated $CD19^+$ B cell counts did not change significantly while $CD3^+CD4^+$, $CD3^+CD8^+$, and $CD3^-CD56^+$ cell counts were considerably lower in individuals with severe illness compared to those with non-severe illness. However, the finding of the current study disagreed with **Wang et al.** [13] who found a non-significant difference in NK cells between both patients groups. However, the severe patients' group had significantly lower B cells count**.**

ROC curve was used to assess the diagnostic value of the lymphocyte subset markers for differentiation of severe and moderate COVID-19 patients, showing that the count of $CD3^+CD4^+$, $CD3^+CD8^+$ T cells and $CD3^-$ $CD56^+$ NK cells are significant predictors of COVID-19 severity with the AUC were 0.884, 0.773 and 0.753 respectively in severe COVID-19 patients, while $CD19^+$ B cell count had no significant relation with disease severity *(p*-value = *0.138)*.

As a result of the highest AUC for $CD3^+CD4^+$ T helper cell count is considered the most important factor in differentiation severe from moderate COVID-19 patients at a cut off value (≤ 264 cell/ uL) with high specificity 100% and sensitivity 66.67% followed by $CD3^+CD8^+$ T cell count with a cutoff value (≤ 195 cell/ uL) with high specificity 93.33% and sensitivity 66.67%. Our findings may shed light on affection of cell mediated immunity with COVID-19 severity. This can be early warning of COVID-19 severity that may help early intervention and treatment.

According to **Luo et al.** [20] The AUCs for different lymphocyte subsets were calculated from ROC curves for predicting severe fatal COVID-19 cases. $CD4^+$, $CD8^+$ T cells and NK cell count were significant predictors of severity with nearly similar AUC (0.906 and 0.905 respectively) for both $CD4^+$ and $CD8^+$ T cells and 0.888 for NK cells. $CD4^+$ and $CD8^+$ T cells were the most significant severity

predictors which agree with our study, However, in contrast to our findings, it has been found that B cells count is a significant positive predictor for disease severity with AUC 0.757.

The current study findings were also compatible with **Bo et al.** [21] who adopted ROC curve to find the warning values of lymphocyte subsets for predicting COVID-19 severity. $CD4^+$ T cell and $CD8^+$ T cell were statistically significant predictors for COVID-19 severity and mortality. The AUC was nearly equal in both (0.82 and 0.81 respectively). On the other hand, $CD19^+$ B cell count was a significant predictor of severity and mortality which disagreed with current study results with AUC 0.70.

IL-6 is an important pro-inflammatory factor in the disease process of SARS-CoV-2. It contributes to COVID-19 associated cytokine storms, largely enhancing vascular permeability and impairing the organs' function. Such an inflammatory response causes inflammation of the respiratory system and other body systems, with subsequent occurrence of ARDS or respiratory failure [22].

The current study clarified that serum concentrations of IL-6 were considerably greater in severe patients than in moderate patients. This study agreed with two **Han et al., and Aziz et al.** [12, 23] both studies concluded that IL-6 level was statistically different among the severe and non-severe patients and that IL6 level was significantly higher among severe patients. However, IL-6 serum level is not significantly correlated with deaths *(p-*value<0.01*)*

The present study revealed that IL-6 serum level could effectively discriminate between moderate and severe patients' group. Using ROC curve analysis, at a cut-off value >60 pg/ml was associated with 80% sensitivity, 86.7% specificity and AUC= 0.84, so IL-6 serum level can be a significant biomarker for prediction of COVID-19 severity *(p- value < 0.001)*.

Aziz et al. [23] and **Sayah and colleagues** [24] had the same results as the current study as regard the use of IL6 serum level in identifying patients at high risk of severe COVID-19 and death**.** Also **El-Shabrawy et al.** [25] found similar results; IL6 serum level seemed to be a valuable marker in predicting severity as well as the prognosis of COVID-19. However, in contrast to this studys' results **Liu et al.** [26] found that IL-6 levels was not associated with COVID-19's mortality.

As regard mortality and relation with lymphocytic count and lymphocytic subsets, the current study shows that patients who died had significant reduction in total lymphocytic count and all T lymphocyte subsets $CD3^+CD4^+$, $CD3^+CD8^+$ and $CD56^+$ cells count than those who survived. However, no significant reduction in $CD19^+$ cells counts was observed.

These results were concordant with **Huang and pranata,** [27] and **Khamiss et al.** [19]. Their results showed that patients with poor outcome had a lower lymphocyte count (*p-value* < 0.001); compared to patients with favorable outcome.

Also **Deng et al.** [14] demonstrated that $CD3^+CD4^+$, $CD3^+CD8^+$ and $CD56^+$ cells counts were significantly lower in patients who died than those who survived. However, $CD19^+$ cells counts didn't differ significantly.

Cantenys-Molina et al. [28] demonstrated that significant reduction of $CD3^+CD4^+$ T cell percentages was associated with mortality and insignificant variations in $CD19^+$ B cell percentages. However, no significant reduction was detected in $CD3^+CD8^+$ T cells and $CD56^+$ NK cell counts which disagreed with our findings.

Conclusion

$CD4^+$ T cells, $CD8^+$ T cells and NK cells were found to be significantly reduced in severe COVID-19 patients than in moderate patients and all can be possible predictors of severity. $CD4^+$ T cell count was the most significant biomarker affected by disease severity with highest specificity and sensitivity. IL-6 serum level can serve as a significant predictor of COVID-19 severity. As regard mortality and relation with lymphocytic count and lymphocytic subsets, total lymphocytic count and all T lymphocyte subsets $CD3^+CD4^+$, $CD3^+CD8^+$ and $CD56^+$ cells count can be used as a significant predictor of death in COVID-19 patients. However, $CD19^+$ cells counts had no relation with death.

Conflicts of interest: Nil.

Financial support and sponsorship: Nil.

Appendix 1

Clinical classification of COVID-19 patients according to disease severity.
Extracted from the Ain Shams Guidelines [12].

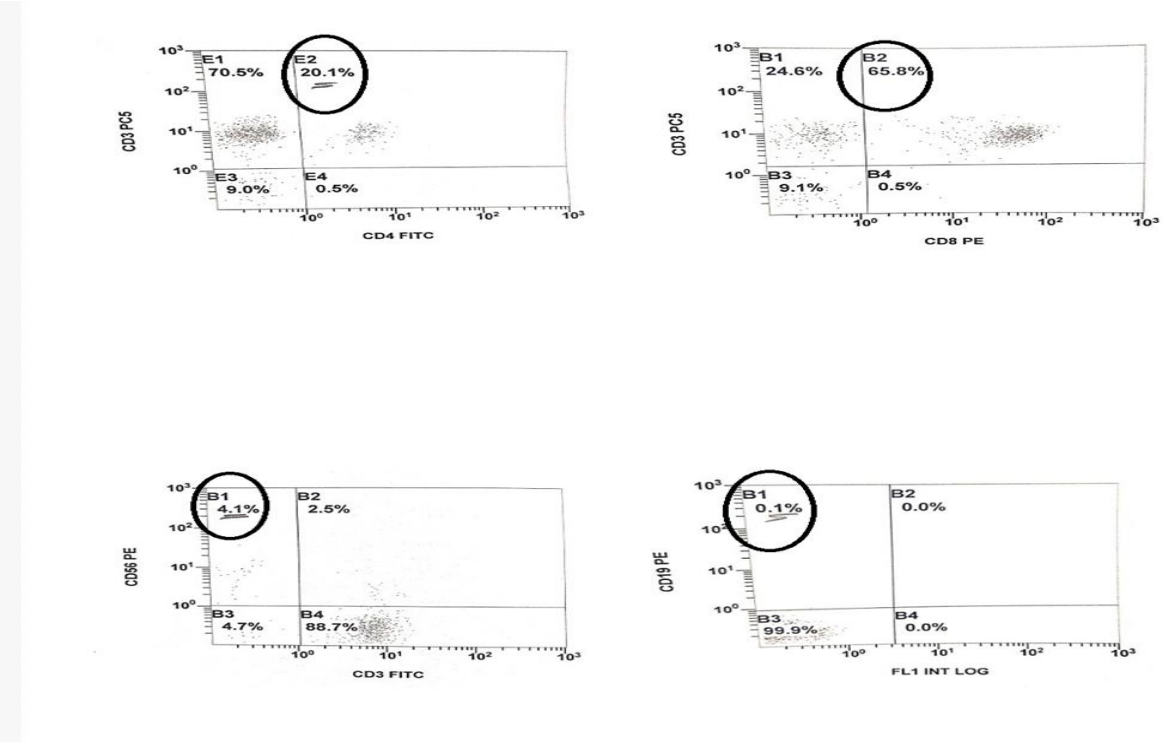
Table 1: COVID-19 disease severity

Mild disease		Symptomatic patients meeting the case definition for COVID-19 without radiological evidence of pneumonia or hypoxia
Moderate disease	Pneumonia	Adolescent or adult with clinical signs of non severe pneumonia (e.g. fever, cough, dyspnea) and radiological evidence of pneumonia
Severe disease	Severe pneumonia	Adolescent or adult with clinical signs of pneumonia (e.g. fever, cough, dyspnea, fast breathing) plus one of the following: • Respiratory rate > 30 breaths/min; severe respiratory distress; orSpO_2 < 93% on room air and radiological evidence of pneumonia • Patients with more than 50% lesions progression within 24 to 48 hours in lung imaging
Critical disease	Acute respiratory distress syndrome (ARDS)	***Meeting any of the following criteria:*** Occurrence of respiratory failure requiring mechanical ventilation; Presence of shock; Sepsis, other organ failure that requires monitoring and treatment in the ICU Critical cases are further divided according to the degree of hypoxemia as categorized by the P/F ratio (PaO2/FiO2 *100) or S/F ratio • Early stage: PO2/FiO2 (P/F ratio) 200-300, or Oxygen saturation by pulse oximetry/ Fraction of inspired oxygen (S/F ratio) 181-235; without organ failure other than the lungs. The patient has a great chance of recovery through active antiviral, anti-cytokine storm, and supportive treatment. • Middle stage: P/F ratio 100-200, or S/F ratio 118-181; may be complicated by other mild or moderate dysfunction of other organs. • Late stage: P/F ratio less than 100, S\F ratio less than 118; diffuse consolidation of both lungs; or failure of other vital organs. The mortality risk is significantly increased. ***Sepsis:*** acute life-threatening organ dysfunction caused by a dysregulated host response to suspected or proven infection. Signs of organ dysfunction include: altered mental status, difficult or fast breathing, low oxygen saturation, reduced urine output, fast heart rate, weak pulse, cold extremities or low blood pressure, skin mottling, laboratory evidence of coagulopathy, thrombocytopenia, acidosis, high lactate, or hyperbilirubinemia. ***Septic shock:*** persistent hypotension despite volume resuscitation, requiring vasopressors to maintain MAP ≥ 65 mmHg and serum lactate level > 2 mmol/L

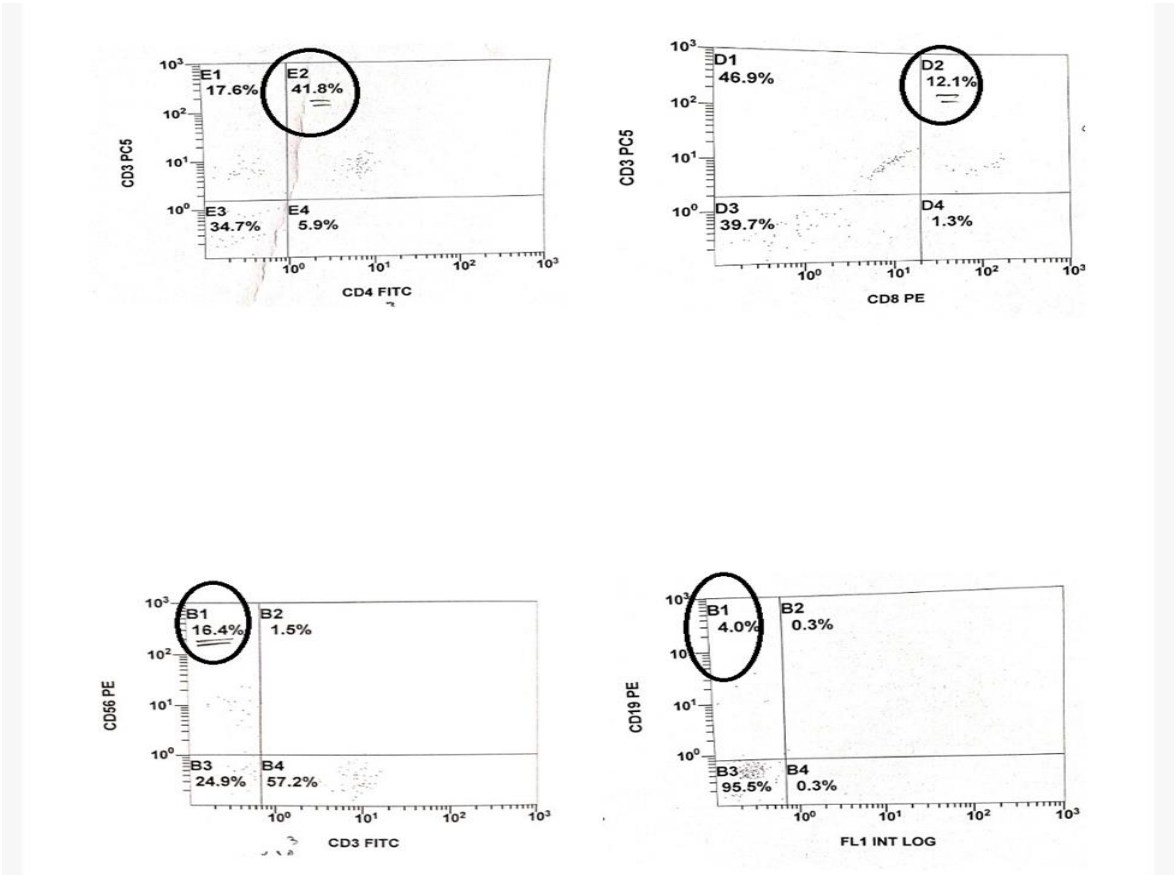
Appendix 2

Flowcytometric representative dot plots from one moderate case (a) and one severe case (b), showing the difference in the peripheral blood $CD3^+CD4^+$, $CD3^+$ $CD8^+$, $CD3^-$ $CD56^+$ and $CD19^+$ cells.

(A) Moderate COVID-19 case



(B) Severe COVID-19 case



References

1-**Zhou P, Yang X, Wang X, Hu B, Zhang L, Zhang W, et al.** A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature 2020; 579(7798):270-273.

2-**Steffens I.** A hundred days into the coronavirus disease (COVID-19) pandemic. Eurosurveillance 2020; 25(14): 2000550.

3-**Guan W, Ni Z, Hu Y, Liang W, Ou C, He J, et al.** Clinical characteristics of coronavirus disease 2019 in China. New England Journal of Medicine 2020; 382(18): 1708–1720.

4-**Yang X, Yu Y, Xu J, Shu H, Liu H, Wu Y, et al.** Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study. The Lancet Respiratory medicine 2020; 8(5): 475–481.

5-**World Health Organization.** WHO situation report 2021(Coronavirus disease (COVID-19) Epidemiological Update).

6- **Herold T, Jurinovic V, Arnreich C, Lipworth B, Hellmuth J, von Bergwelt-Baildon M, et al.** Elevated levels of IL-6 and CRP predict the need for mechanical ventilation in COVID-19. Journal of Allergy and Clinical Immunology 2020; 146(1): 128-136.e4.

7-**Johnson K, Harris C, Cain J, Hummer C, Goyal H, Perisetti A.** Pulmonary and extra-pulmonary clinical manifestations of COVID-19. Frontiers in Medicine 2020; 7: 526.

8-**Zheng Z, Peng F, Xu B, Zhao J, Liu H, Peng J, et al.** Risk factors of critical & mortal COVID-19 cases: A systematic literature review and meta-analysis. Journal of Infection 2020; 81(2):e16-e25.

9-**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; 181(2): 271-280.e8.

10-**Qin C, Zhou L, Hu Z, Zhang S, Yang S, Tao Y, et al.** Dysregulation of immune response in patients with COVID-19 in Wuhan, China. Clinical Infectious Diseases 2020; 71(15):762-768.

11-**Coperchini F, Chiovato L, Croce L, Magri F, Rotondi M.** The cytokine storm in COVID-19: An overview of the involvement of the chemokine/chemokine-receptor system. Cytokine and Growth Factor Reviews 2020; 53:25-32.

12-**Han H, Ma Q, Li C, Liu R, Zhao L, Wang W, et al.** Profiling serum cytokines in COVID-19 patients reveals IL-6 and IL-10 are disease severity predictors. Emerging Microbes and Infections 2020; 9(1): 1123–1130.

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. The Journal of Infectious Diseases 2020; 221(11): 1762–1769.

14-**Deng Z, Zhang M, Zhu T, Zhili N, Liu Z, Xiang R, et al.** Dynamic changes in peripheral blood lymphocyte subsets in adult patients with COVID-19. International Journal of Infectious Diseases 2020; 98: 353–358.

15-**Lab BT.** Human Interleukin 6 ELISA kit Cat. No E0090Hu Available at: https://www.bt-laboratory.com/index.php/Shop/Index/productShijiheDetail/p_id/253

16-**Bahadur S, Long W, Shuaib M.** Human coronaviruses with emphasis on the COVID-19 outbreak. Virus Disease 2020; 31(2):1-5.

17-**Qian F, Gao G, Song Y, Xu Y, Wang A, Wang S, et al.** Specific dynamic variations in the peripheral blood lymphocyte subsets in COVID-19 and severe influenza A patients: a retrospective observational study. BMC infectious diseases 2020; 20(1): 1-11.

18-**Zhang W, Li L, Liu J, Chen L, Zhou F, Jin T, et al.** The characteristics and predictive role of lymphocyte subsets in COVID-19 patients. International Journal of Infectious Diseases 2020; 99: 92-99.

19-**Khamiss A, El-Dahshan M, El-Ghamery F, Aggag M, Hashim A, Eliwa A.** Outcomes of COVID-19 in Egyptian patients. Al-Azhar Medical Journal 2021; 50(1): 765-782.

20-**Luo Y, Mao L, Yuan X, Xue Y, Lin Q, Tang G, et al.** Prediction model based on the combination of cytokines and lymphocyte subsets for prognosis of SARS-CoV-2 infection. Journal of clinical immunology 2020; 40(7): 960-969.

21-**Bo X, Fan C, Wang A, Zou Y, Yu Y, Cong H, et al.** Suppressed T cell-mediated immunity in patients with COVID-19: a clinical retrospective study in Wuhan, China. Journal of Infection 2020; 81(1): e51-e60.

22-**Bhandari S, Rankawat G, Singh A, Wadhwani D, Patel B.** Evaluation of Interleukin - 6 and its Association with the Severity of Disease in COVID - 19 Patient. Indian J Med Spec 2020; 11:132-6.

23-**Aziz M, Haghbin H, Lee-Smith W, Goyal H, Nawras A, Adler D.** Gastrointestinal predictors of severe COVID-19: systematic review and meta-analysis. Annals of Gastroenterology 2020; 33(6): 615.

24-**Sayah W, Berkane I, Guermache I, Sabri M, Lakhal F, Rahali S, et al.** Interleukin-6, procalcitonin and neutrophil-to-lymphocyte ratio: Potential immune-inflammatory parameters to identify severe and fatal forms of COVID-19. Cytokine 2021; 141: 155428.

25-**El-Shabrawy M, Alsadik M, El-Shafei M, Abdelmoaty A, Alazzouni A, Esawy M, Shabana M.** Interleukin-6 and C-reactive protein/albumin ratio as predictors of COVID-19 severity and mortality. The Egyptian Journal of Bronchology 2021; 15(1): 1-7.

26-**Liu X, Wang H, Shi S, Xiao J.** Association between IL-6 and severe disease and mortality in COVID-19 disease: a systematic review and meta-analysis. Postgraduate Medical Journal 2021

27-**Huang I, Pranata R.** Lymphopenia in severe coronavirus disease-2019 (COVID-19): systematic review and meta-analysis. Journal of intensive care 2020; 8: 1-10.

28-**Cantenys-Molina S, Fernández-Cruz E, Francos P, Lopez Bernaldo de Quirós J, Muñoz P, Gil-Herrera J**. Lymphocyte subsets early predict mortality in a large series of hospitalized COVID-19 patients in Spain. Clinical and Experimental Immunology 2021; 203(3): 424-432.

Abd Elhady M, Abd Elrahman AT, Elsheikh N, Salah El-Deen NN. Evaluation of peripheral lymphocyte subsets' alteration and IL6 serum level correlated with severity and outcome in corona virus disease 2019 (COVID-19). Microbes Infect Dis 2022; 3(1): 24-35.