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Dynamic Changes of Lymphocyte Subsets in the Course of COVID-19

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Keywords
Coronavirus disease 2019 · Cellular immunity · Lymphocyte · Flow cytometry

Abstract
Background: Although the pathophysiology of coronavirus disease 2019 (COVID-19) is not clearly defined, among the proposed mechanisms, immune system dysfunction is more likely than others. The aim of this study was to clarify the characteristics and clinical significance of dynamic changes of lymphocyte subsets in the course of COVID-19. ***Methods:*** In this prospective study, the levels of peripheral lymphocyte subsets including $CD4^+$, $CD8^+$, $CD4^+CD25^+FOXP3^+$, $CD38^+$, $CD3^+HLA\text{-}DR^+$, $CD19^+$, $CD20^+$, and $CD16^+CD56^+$ cells were measured by flow cytometry in 52 confirmed hospitalized patients with COVID-19 at the day of admission and after 7 days of care. Clinical response was defined as improvement in symptoms (fever, dyspnea, and cough as well as blood oxygen saturation), and patients who met these criteria after 1 week of admission were classified as early responders; others who survived and finally discharged from the hospital were classified as late responders and patients who died were categorized as nonresponders. Immunophenotyping of studied cell changes on the first day of admission and 7 days after treatment were compared. Besides, the correlation between cellular subset variation and clinical response and outcome were analyzed. ***Results:*** Total counts of white blood cell, T cells, $CD4^+$ T cells, $CD8^+$ T cells, $CD38^+$ lymphocytes, and $CD3^+HLA\text{-}DR^+$ lymphocytes were significantly increased in both early and late responders. No statistically significant difference was observed in $CD4^+/CD8^+$ ratio, B cells, $FOXP3^+T_{reg}$ lymphocytes, and FOXP3 median fluorescence intensity among studied groups. According to the multivariate analysis, an increase in $CD4^+$ T cells ($p = 0.019$), $CD8^+$ T cells ($p = 0.001$), and administration of interferon ($p < 0.001$) were independent predictors of clinical response. ***Conclusion:*** We found an increasing trend in total T cells, T helpers, cytotoxic T cells, activated lymphocytes, and natural killer cells among responders. This trend was not statistically significant among nonresponders. The findings of this study may enhance our knowledge about the pathogenesis of COVID-19.

Introduction

The coronavirus disease 2019 (COVID-19) is caused by a novel coronavirus officially designated as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The first cases occurred in December 2019 in China, and

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then the virus spread rapidly in numerous countries throughout the world [1, 2]. It has led to a public health emergency of international concern, and the World Health Organization on January 30, 2020, introduced COVID-19 as a pandemic disease [3].

The pathophysiology of COVID-19 has not yet been clearly defined [4]. Existing data from severe forms of Middle East respiratory syndrome coronavirus and SARS-CoV and recent accumulating evidence concerning SARS-CoV-2 suggest that the host immunity response is contributing to the pathogenesis of COVID-19 [2, 5, 6].

Lymphocytes in peripheral blood including T cells, B cells, and natural killer (NK) cells are involved in the humoral and cellular immunity against viral infections [7], and effective immune response against a viral infection is dependent on the activation of cytotoxic $CD8^+$ T cells through the killing of virus-infected cells [8]. Also, regulatory T cells (T_{reg}) lymphocytes have an important role in the prevention of excessive and harmful immune reactions to the pathogens [4]. Thus, a better understanding of the characteristics of cellular immunity in COVID-19 could provide novel insights into the pathogenesis of the disease. An increasing number of studies concerning changes of lymphocyte subsets, such as $CD4^+$ T cells, $CD8^+$ T cells, B cells, and NK cells in COVID-19 patients and their correlation with the severity and outcome of the disease have been performed [9–11], but our knowledge about dynamic of lymphocyte subsets during the course of the disease is limited to few studies [12, 13]. Moreover, kinetic changes in cellular response may be correlated to clinical outcome. Therefore, to obtain a clearer picture of this entity, we designed a prospective, single-center study. In this study, we aimed to clarify the characteristics and clinical significance of changes of lymphocyte subsets during the course of COVID-19 and determine if these changes are associated with the prognosis, which might help in better understanding of the pathogenesis of the disease and lead to the discovery of new therapeutic options.

Materials and Methods

The Ethics Committee of the National Research Institute of Tuberculosis and Lung Diseases approved the protocol of this study (reference number: IR.SBMU.NRITLD.REC.1399.037IR.SBMU.NRITLD.REC.1399.037). Also, informed consent was obtained from all the participants.

Patients

This prospective study was conducted at Masih Daneshvari Hospital, Tehran, Iran. In the period of study, confirmed cases of COVID-19 who were symptomatic and admitted to the infectious disease ward were recruited to the study. Patients with a history of autoimmune disease, immunocompromised state, or immune suppression therapy were excluded. The presence of SARS-CoV-2 was detected by the real-time reverse transcription PCR (RT-PCR) method using a nasopharyngeal swab, as previously described [14]. Two blood samples were obtained from all patients, one on the first day of admission, and the second after 7 days of care.

Concerning the severity of the disease, the patients were categorized into 3 groups: moderate, severe, and critical. At rest, oxygen saturation in room air at the time of sampling was used to determine the severity of the disease. Patients with abnormal chest imaging and O_2 saturation >93% were categorized as cases with moderate disease. Cases with O_2 saturation equal or <93% were classified as severe cases, and patients who needed critical care, noninvasive or mechanical ventilation, or in a shock state were categorized as critical cases [15].

Therapeutic Interventions

All the patients received supportive care, supplemental oxygen, and intravenous fluid. Considering the treatment protocol released by the Iranian national scientific committee for management of COVID-19, lopinavir/ritonavir 400/100 mg was prescribed for 7 days [16]. Also, 3 doses of subcutaneous interferon beta-1a (12 million units) every other day were used for some cases and intravenous dexamethasone (4 mg BID) for limited patients.

Assessment of Clinical Response

Clinical response was defined as improvement in symptoms (fever, dyspnea, and cough) as well as blood oxygen saturation. Patients who met these criteria after 1 week of admission were classified as early responders; others who survived and finally discharged from the hospital were classified as late responders and patients who died were categorized as nonresponders.

Method of Flow Cytometry

In both sampling, 2 mL of whole blood was obtained, collected in tubes containing EDTA, and tested up to 6 h after sampling. Total cell blood counts and percentage and absolutes counts (cells/μL) of $CD3^+$, $CD4^+$, and $CD8^+$ T lymphocytes; $CD19^+$ and $CD20^+$ B lymphocytes; $CD16^+$ $CD56^+$ NK cells; and $CD4^+CD25^+FOXP3^+T_{reg}$ were measured.

For this purpose, after centrifuging of the samples, RBCs were removed using lysis buffer. Then white blood cells (WBCs) were harvested and washed with cold PBS. Before staining, the cell-surface Fc receptors were blocked with 2.4G2 (PharMingen, San Diego, CA, USA). Phycoerythrin-conjugated anti-human CD4, CD19, and CD56 antibodies (PharMingen) were used to stain $CD4^+$ T cells, $CD19^+$ B cells, and $CD56^+$ NK cells. Allophycocyanin-conjugated anti-human CD8 and CD16 antibodies were used to stain $CD8^+$ and $CD16^+$ cells; and fluorescein isothiocyanate-conjugated antibody for $CD3^+$ T cells according to the manufacturer's instructions. The cells were analyzed on a FACSCalibur™ flow cytometer (Becton Dickinson, San Jose, CA, USA). Dead cells were gated out by staining with propidium iodide. FlowJo software version 8 was used for the final analysis.

Statistical Analysis

Categorical variables were described as number and percentage, and continuous variables, as median and interquartile

range. Categorical data were compared using the χ^2 test or Fisher's exact test. The Kolmogorov-Smirnov and Shapiro-Wilk tests were used to test the normality of data. For comparison of medians between different groups of severity, the Kruskal-Wallis test was used and differences between the 2 groups were analyzed using the Mann-Whitney test. SPSS Statistics version 21.0 software was used for statistical analyses. All reported p values are two sided and a value of <0.05 was considered statistically significant. The generalized estimating equation model was used to estimate and find how factors affect the response variable as a crude and adjusted model.

Immunophenotyping of studied cell changes on the first day of admission and 7 days after treatment were compared. Besides, the correlation between cellular subset variation and clinical response were analyzed.

Results

Demographics and Baseline Characteristics

From June 8 to June 15, 2020, 52 confirmed COVID-19 cases were recruited in this study. Infection with SARS-CoV-2 was confirmed by RT-PCR. The mean age of the cases was 51.3 ± 14.8 years, including 24 men (46.2%) and 28 women. Among them, 51.9% had minimally 1 comorbidity; diabetes mellitus (26.9%), and hypertension (19.2%) were the most common. The median time from onset of symptoms to first sampling was 10.4 ± 5.2 days. Twenty-seven (51.9%) and 8 cases (15.4%) were categorized as experiencing severe illness and a critical state on admission. Demographics, basic characteristics, treatment, and the outcome of the patients are listed in Table 1.

Based on the clinical response, the patients were divided into 3 groups: early responders (30 cases, 57.7%), late responders (18 cases, 34.6%), and nonresponders (4 cases, 7.7%). All 3 groups were similar concerning age, gender, duration from onset of symptom, and steroid therapy. There was a significant correlation between the severity of the disease and the clinical response (p < 0.0001). All the patients with moderate disease, as well as 13 cases with severe disease (48.1%) on admission, had a clinical response on the seventh day. Twenty-two patients had a stable condition (no improvement and no deterioration) after 1 week, among them, 18 cases finally survived and 4 died.

Dynamic of Cellular Subsets in the Course of the Disease

We compared WBC and lymphocyte subset counts at days 0 of admission and following 1 week posttreatment for all patients. Table 2 shows the distribution of WBC and lymphocyte subsets between days 0 and 7 of admission and the significance of their difference in detail. Except for T_{reg} T cells, B cells, and FOXP3$^+$ median fluorescent intensity (MFI), other parameters were significantly different between the 2 times. Moreover, the CD4$^+$/CD8$^+$ ratio did not statistically change during the study time. WBC, relative and absolute count of lymphocytes, absolute count of T cells, CD4$^+$ T cells, CD8$^+$ T cells, CD27$^+$ lymphocytes, CD3$^+$HLA DR$^+$ lymphocytes, CD38$^+$ lymphocytes, and NK cells were significantly increased. In addition, the relative count of polymorphonuclears after 7 days of treatment was decreased. Change in cellular subsets among patients under interferon therapy was limited to WBC, total lymphocyte, and T cells (see online suppl. Table 1; for all online suppl. material, see www.karger.com/doi/10.1159/000514202).

Table 1. Demographic and clinical characteristics of 52 confirmed cases of COVID-19

Parameter	*N* (%)
Age	
Mean ± SD, years	51.3±14.8
Gender	
Male	24 (46.2)
Female	28 (53.8)
Comorbidity	27 (51.9)
Diabetes mellitus	14 (26.9)
Hypertension	10 (19.2)
Chronic heart diseases	6 (11.5)
Chronic obstructive lung diseases	3 (5.8)
BMI ≥30	4 (7.7)
Other risk factors	3 (5.8)
From the onset of illness to 1st sample	
Mean ± SD, days	10.4±5.2
Severity at admission	
Moderate	17 (32.7)
Severe	27 (51.9)
Critical	8 (15.4)
Treatment modality	
Lopinavir/ritonavir	52 (100)
Interferon	19 (36.5)
Steroid	6 (11.5)
Clinical response and outcome	
Cure	48 (92.3)
Early response	30 (57.7)
Late response	18 (34.6)
Death	4 (7.7)
Total	52

COVID-19, coronavirus disease 2019.

Table 2. Comparison of WBC and lymphocyte subsets between day 0 and 7

Parameter*	Day 0		Day 7		*p* value
	median	IQR	median	IQR	
WBC	5,880	(4,702–8,417)	7,130	(5,230–9,755)	0.001**
PMN, %	63.5	(53.0–73.3)	56.4	(48.3–67.5)	0.004**
Lymphocyte, %	25.1	(17.3–31.3)	30.0	(22.3–37.3)	0.019**
Total lymphocyte	1,416	(1,035–1,936)	1,808	(1,498–2,391)	<0.0001**
T cells	863	(551–1,237)	1,255	(932–1,548)	<0.0001**
$CD4^+$ T cells	598	(347–821)	787	(576–920)	<0.0001**
$CD8^+$ T cells	256	(141–357)	394	(248–478)	<0.0001**
$CD4^+/CD8^+$ (ratio)	2.7	(1.6–3.4)	2.2	(1.5–3.2)	0.132
T_{reg} cells#	49	(6–108)	55	(0–186)	0.286
$FOXP3^+$ MFI#	6.5	(5.1–14.5)	4.2	(3.6–14.1)	0.859
$CD27^+$ lymphocytes	275	(126–507)	344	(202–611)	0.003**
$CD38^+$ lymphocytes	166.5	(112.5–225.5)	273.4	(196.6–370)	<0.0001**
$CD3^+HLA\ DR^+$	44.3	(0–86)	82.9	(9.6–216)	0.003**
$CD19^+$ B cells	146	(87–300)	184	(100–304)	0.189
$CD20^+$ B cells	136	(85–280)	171	(102–284)	0.188
NK cells	201	(149–302)	265	(183–380)	0.014**

IQR, interquartile range; WBC, white blood cell; PMN, polymorphonuclear; T_{reg}, regulatory T lymphocyte ($CD3^+CD25^+FOXP3^+$); MFI, median fluorescent intensity; NK, natural killer. * All parameters are counts (cells per cubic millimeter) other than specified. ** Statistically significant. # FOXP3 marker was checked for 31 samples on the 1st day and 11 samples on the 7th day.

Cellular Subsets and Outcome of the Disease

Patients who finally died had a higher neutrophil percentage ($p = 0.013$), lower lymphocyte percentage ($p = 0.032$), and lower counts of total T cells, $CD4^+$ and $CD8^+$ T cells, and NK cells at day 0 of admission. On the seventh day of hospitalization, in addition to the above factors, lower counts of $CD27^+$ and $CD3^+HLA\ DR^+$ lymphocytes were associated with death (online suppl. Tables 2, 3). After using multiple adjusted logistic regression analysis, interferon therapy was the only parameter associated with early response and none of the parameters was a predictor of mortality (online suppl. Tables 4, 5).

Dynamic Changes in Cellular Subsets concerning Clinical Response

Table 3 indicates the detailed dynamic changes of the WBC and lymphocyte subsets according to the clinical response. Total counts of WBC, T cells, $CD4^+$ T cells, $CD8^+$ T cells, $CD3^+$ $HLA\ DR^+$ lymphocytes, and $CD38^+$ lymphocytes were significantly increased in both early and late responders. An increase in the counts of $CD27^+$ lymphocytes and NK cells was correlated with early response. No statistically significant difference was observed in $CD4^+/CD8^+$ ratio, B cells, $FOXP3^+T_{reg}$ lymphocytes, and FOXP3 MFI among studied groups.

Dynamic Changes in Cellular Subsets concerning the Outcome

Four cases eventually died, all of them were nonresponders at the time of the second sampling. A significant increase of total lymphocyte count, T cells, $CD4^+$ T cells, $CD8^+$ T cells, $CD27^+$ lymphocytes, $CD3^+HLA\ DR^+$ lymphocytes, and NK cells were seen among patients who survived. This trend was not statistically significant among the nonresponders (death group). There was no significant difference in the $CD4^+/CD8^+$ ratio, counts of B cells, and $FOXP3^+T_{reg}$ cells, as well as FOXP3 MFI.

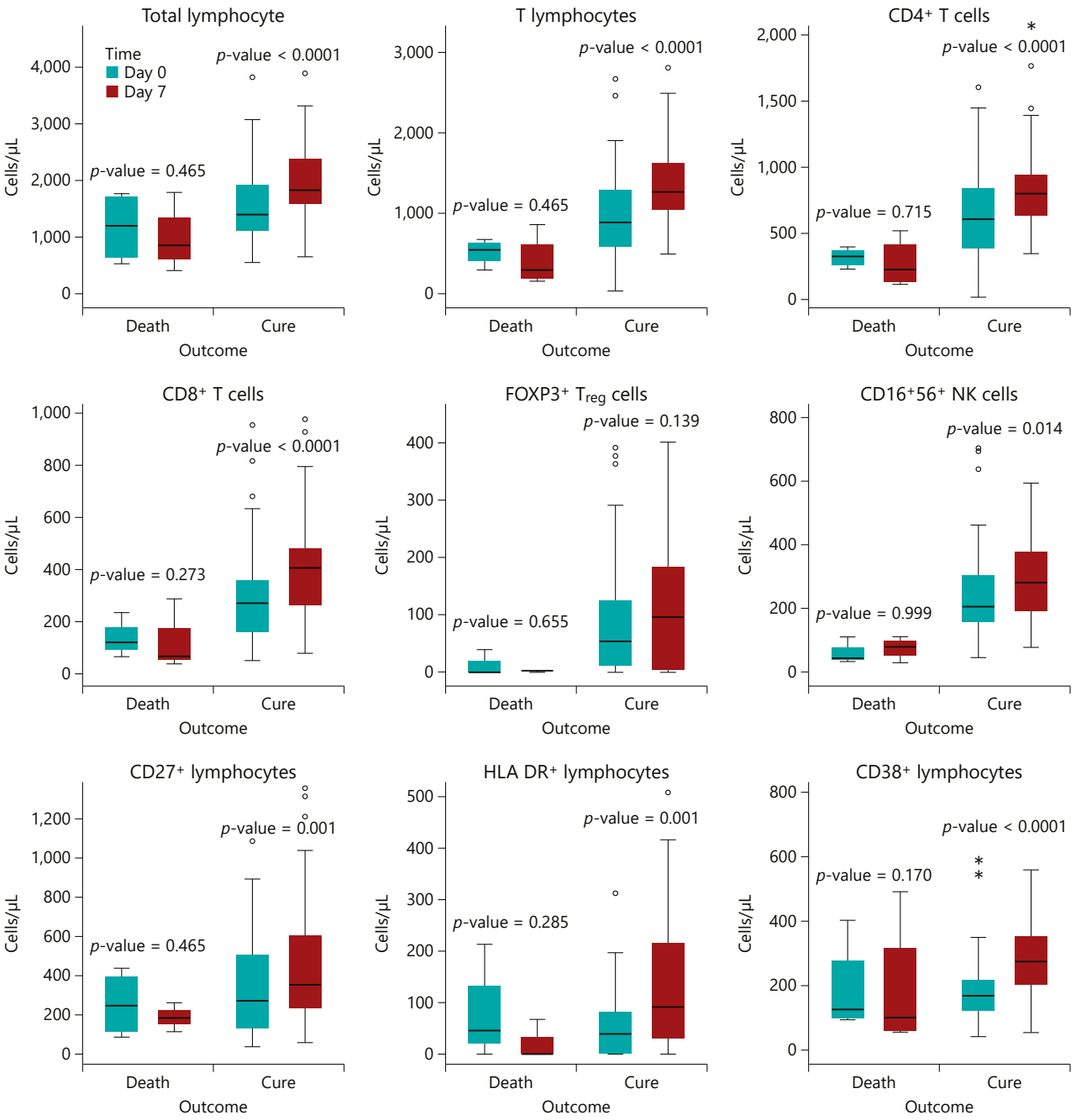
The most important findings of the correlation between cellular subsets and outcome were shown in Figure 1.

According to the multivariate analysis, an increase in $CD4^+$ T cells ($p = 0.019$) and $CD8^+$ T cells ($p = 0.001$) both after 1-week posttreatment, and administration of interferon ($p < 0.001$) as independent predictors of clinical response, with adjusting the factors of age, sex, total lymphocyte count, and counts of NK cells was found (Table 4).

Table 3. Comparison of WBC and lymphocyte subsets concerning clinical response

	Early response ($N = 30$)			Late response ($N = 18$)			No response ($N = 4$)		
	Median	IQR	*p* value	median	IQR	*p* value	median	IQR	*p* value
WBC									
Day 0*	5,605	(4,710–8,560)	0.019**	6,375	(4,580–7,160)	0.028**	7,600	(4,870–14,280)	0.144
Day 7*	6,640	(5,220–9,010)		7,175	(5,160–10,780)		10,190	(7,545–18,930)	
PMN, %									
Day 0	60.4	(51–66)	0.012**	66.5	(59–74.2)	0.028**	81	(71.9–86.7)	0.068
Day 7	51.7	(46–59.5)		60	(56.1–65)		87.2	(85–90.4)	
Lymphocyte, %									
Day 0	27	(21.7–36)	0.021**	24	(18–31)	0.055	12.5	(8.65–21.05)	0.068
Day 7	32.8	(28–38.2)		25.8	(22.5–33.1)		7.3	(6.5–8.7)	
Total lymphocyte									
Day 0	1,589.5	(1,080–2,198)	0.004**	1,375.5	(1,110–1,733)	0.001**	1,198	(636–1,719.5)	0.465
Day 7	1,955.5	(1,712–2,652)		1,728	(1,331–1,953)		869.5	(602–1,362.5)	
T cells									
Day 0	1,020.5	(659–1,428)	0.003**	856	(355–951)	0.001**	547	(396–637)	0.465
Day 7	1,338.5	(1,129–1,910)		1,224	(799–1,370)		295.5	(187–617)	
CD4+ T cells									
Day 0	678.5	(383–926)	0.006**	598.5	(312–680)	0.005**	326	(260–381.5)	0.715
Day 7	803.5	(707–1,226)		756.5	(527–914)		228	(130–416)	
CD8+ T cells									
Day 0	310.5	(179–421)	0.004**	197	(145–272)	0.005**	119.5	(90.5–178)	0.273
Day 7	438.5	(343–585)		322	(221–422)		65	(50–176)	
CD4/8 ratio									
Day 0	2.4	(1.4–3.3)	0.309	2.8	(2.2–4.2)	0.199	3.2	(2.1–3.5)	0.715
Day 7	2.2	(1.4–2.8)		2.3	(1.5–3.3)		2.6	(1.9–4.1)	
T_{reg} T cells#									
Day 0	72	(22–108)	0.465	20	(4–144)	0.225	0	(0–39)	0.655
Day 7	29	(1.5–103)		186	(96–194)		2.5	(0–5)	
$FOXP3^+$ MFI#									
Day 0	8	(5.5–14.8)	0.465	6.5	(5.3–14.5)	0.500	4.1	(4–4.6)	0.180
Day 7	4.15	(2.95–5.95)		14.1	(6.5–16.8)		3.65	(3.6–3.7)	
$CD27^+$ lymphocytes									
Day 0	259	(126–533)	0.006**	282	(127–408)	0.058	247.5	(110.5–399)	0.465
Day 7	437	(239–712)		299.5	(200–396)		185	(147.5–226.5)	
$CD38^+$ lymphocytes									
Day 0	171	(125–227)	0.011**	168	(116–193)	0.006**	126.5	(97–278)	0.715
Day 7	253.4	(195–366)		307.2	(236.2–373.9)		100.9	(56.3–317.1)	
$CD3^+HLA\ DR^+$									
Day 0	30.8	(0–84.2)	0.011**	50.7	(0–100)	0.044**	45.3	(18.3–133.5)	0.285
Day 7	76.9	(19.3–168.1)		189.7	(52.5–265.8)		0	(0–33.7)	
$CD19^+$ B cells									
Day 0	141.5	(87–319)	0.111	152	(105–237)	0.906	229	(56–408)	0.715
Day 7	197	(150–315)		133	(88–205)		231	(95–400)	
$CD20^+$ B cells									
Day 0	129.5	(85–312)	0.102	152	(104–237)	0.723	228.5	(49–408)	1.000
Day 7	188	(149–284)		124	(84–205)		223.5	(92.5–395.5)	
NK cells									
Day 0	221	(171–338)	0.045**	166	(153–279)	0.193	43	(33–110)	0.999
Day 7	309	(246–411)		218.5	(137–298)		79	(49.5–100)	

IQR, interquartile range; WBC, white blood cell; PMN, polymorphonuclear; T_{reg}, regulatory T lymphocyte ($CD3^+CD25^+FOXP3^+$); MFI, median fluorescent intensity; NK, natural killer. * Day of hospitalization. ** Statistically significant. # FOXP3 marker was checked for 31 samples on the 1st day and 11 samples on the 7th day.



Color version available online

Fig. 1. Relation between the outcome of COVID-19 and dynamic changes of cellular subsets between day 0 and 7 of admission p value <0.05 is significant. COVID-19, coronavirus disease 2019; T_{reg}, regulatory T lymphocyte ($CD3^+CD25^+FOXP3^+$); NK, natural killer.

Table 4. Multiple analysis of dynamic changes of cellular subsets between day 0 and 7 of admission and early clinical response

Parameters	*p* value	Odds ratio	95% CI
Older age	0.129	1.055	0.985–1.131
Male	0.063	0.142	0.018–1.107
Total lymphocyte increase	0.549	1.000	0.999–1.002
$CD4^+$ T cells increase	0.027*	1.004	1.000–1.008
$CD8^+$ T cells increase	0.004*	0.989	0.981–0.996
CD4/8 ratio increase	0.074	0.292	0.076–1.127
NK cell increase	0.212	0.992	0.979–1.005
Interferon therapy	<0.001*	70.886	9.649–518.345

* Statistically significant. (*p* value <0.05). NK, natural killer; CI, confidence interval.

Discussion

Since December 2019, when COVID-19 was first reported from China, numerous studies were published to clarify the pathogenesis of the disease and various hypotheses have been proposed in this regard [17–19]. Among the proposed mechanisms underlying the worsening of the disease, the strongest is immune system dysfunction [20]. Recent findings hypothesize that virus-induced immune system dysregulation can lead to T cell exhaustion [4, 21] and cytokine storm [22–24]. Lymphocyte and the subsets play an important role in the performance of the adaptive immune system and flow cytometry is a valuable tool for monitoring the maintenance of cellular immunity [25, 26]. A growing list of studies regarding lymphocyte subset counts among patients with COVID-19 has been published, most of them focusing on the prognostic value and correlation of cellular subsets with disease severity among COVID-19 patients [10, 11, 27–30]. However, a limited number of studies have concentrated on the dynamic and longitudinal changes of these parameters during the course of COVID-19 [12, 13, 31, 32]. Available data indicates that lymphopenia, particularly in $CD4^+$ and $CD8^+$ T cells is common during SARS-CoV-2 infection [2, 33] especially in severe or critical cases [34, 35].

In this study, we found a significant increasing trend in the counts of WBC, total lymphocytes, total T cells, $CD4^+$ T cells, and $CD8^+$ T cells, and the improvement of COVID-19. This correlation was observed among patients with both the early (in the first week of admission) and late (after 7 days of admission) clinical response. These results are consistent with the work of Moratto et al. [12] in Italy. They evaluated the longitudinal values of lymphocyte subsets in 26 patients with COVID-19 and observed improvement in the counts of $CD3^+$, $CD4^+$, and $CD8^+$ cells among patients who eventually recovered, while in cases who progressed to a worse condition, all of these subsets remained very low. Also, Weiskopf and colleagues performed a study to evaluate the kinetics of cellular immunity response in 19 patients with COVID-19 during the acute phase of respiratory distress syndrome. They showed that SARS-CoV-2 specific T cells (both $CD4^+$ and $CD8^+$) appeared during 2 weeks after onset of the symptoms, and a direct negative correlation between viral loads and $CD4^+$ T cells was found [32].

Wang et al. [13] assessed dynamic changes of $CD4^+$ cells, $CD8^+$ cells, B cells, and NK cells in a 1-week interval in 60 patients with COVID-19. They detected no significant changes in any lymphocyte subset in nonresponder patients. In responder group patients, total lymphocytes, $CD8^+$ T cells, and B cells increased significantly after 7, and no statistically significant alteration was found in $CD4^+$ T cells, $CD4^+/CD8^+$ ratio, and NK cells. Also, Huang and coworkers showed that T lymphocytes (total, $CD4^+$, and $CD8^+$ cells) had a downward trend until the fourteenth day and then gradually returned to the normal levels except in the unimproved patients with COVID-19. They concluded that irreversible damage of cellular immunity occurs in the early stages of COVID-19 in the unimproved group [36]. In a similar work, Deng et al. [37] measured lymphocyte subset counts weekly and found a decreasing trend during the first week, while a stable trend was seen during the second week after the onset of symptoms. After that, these markers increased gradually and rose to the normal levels in the fifth week although they were lower in patients who finally died.

Our results showed an association between the decreasing trend of neutrophil percentage and clinical response. This finding is consistent with the work of Wang et al. [38] They showed that excessive neutrophils were associated with disease severity.

In the present study, patients with good outcomes including early and late responders displayed a similar trend in the lymphocyte subset. These findings are consistent with the work published recently by Mathew and coworkers [31]. They found that some patients with COVID-19 had dynamic changes in the lymphocyte subset during the first week of admission, but others remained stable; this finding confirms the different immune responses among the patients [31]. On the other hand, hospitalized patients are in different phases in the course of

the disease. Therefore, more frequent measurement of the elements (e.g., sampling every week) until normalization gives us more information.

HLA DR$^+$ and CD38$^+$ T cells are the markers of T lymphocyte activation. Although the total lymphocyte counts and the subsets decrease in COVID-19, deep analysis of T cells revealed a correlation between the activation of T cells and antiviral responses [31]. In our study, CD3$^+$ HLA DR$^+$ and CD38$^+$ lymphocytes were significantly increased during the first week of admission in responders.

T_{reg} cells are essential for immune homeostasis. They suppress autoimmune reactions and inhibit exaggerated inflammatory responses following viral infections [39]. The available evidence suggests that the T_{reg} count in peripheral blood is reduced in patients with COVID-19 especially in severe cases [9, 28, 40]. The reason for this phenomenon is not clearly defined and may be one of the causes of immune system hyperactivation that lead to lung damage in the severe form of COVID-19 [41]. We did not find any significant differences concerning the percent and count of T_{reg} T cells and FOXP3 MFI during the first week. Frequent measurements can give us more accurate information about the condition of the T_{reg} in the course of the disease. Since the T_{reg}-based therapies had been proposed as a potential tool for control of the severe form of COVID-19 [41, 42], more investigation about the role and kinetics of T_{reg} is recommended.

Our data about the role of NK cells in the pathogenesis of COVID-19 are limited. In one study there were no differences in the levels of NK cells among responders and nonresponders before and following the treatment [13]. In another study, this trend was increasing among survivors and decreasing among non-survivors [37]. In our study, increasing of NK cells during the first week of admission correlated with clinical response.

Some of the treatments experimentally used in the management of COVID-19 might affect total lymphocyte counts and the subsets. For example, corticosteroids have lymphocytolytic effects and could reduce the lymphocytes, while in one study corticosteroid treatment of patients with COVID-19 increased the total number of lymphocytes significantly [13]. In our study, 19 patients received interferon beta-1a that may cause cytopenia [43]. In multivariate analysis, we showed a posttreatment increase in CD4$^+$ and CD8$^+$ T cell counts as predictors of good response, after adjusting the effects of other factors.

Conclusion

In the current study after a follow-up of the dynamic changes of lymphocyte subsets among COVID-19 patients, we found an increasing trend in WBC, total T cells, CD4$^+$ T cells, CD8$^+$ T cells, CD38$^+$ lymphocytes, and CD3$^+$HLA DR$^+$ lymphocytes among responders. This trend was not statistically significant among nonresponders. The finding of this study may enhance our knowledge about the pathogenesis of COVID-19 that could help to design therapeutic options for control of severe cases.

Statement of Ethics

The research was conducted ethically in accordance with the World Medical Association Declaration of Helsinki. The Ethics Committee of the National Research Institute of Tuberculosis and Lung Diseases approved the protocol of this study (reference number: IR.SBMU.NRITLD.REC.1399.037IR.SBMU.NRITLD.REC.1399.037). Also, informed consent was obtained from all participants.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

M.R. and M.M. had the idea for and designed the study and had full access to all data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. M.R. and S.M. contributed to the writing of the manuscript. All authors contributed to data acquisition, data interpretation, and reviewed and approved the final version.

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