

# Reduced Counts of Various Subsets of Peripheral Blood T Lymphocytes in Patients with Severe Course of COVID-19

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This study was intended to define T lymphocyte subsets in different clinical groups of COVID-19-infected patients to explore the interaction between T cell-mediated immune response and the severity of COVID-19 course. Lymphopenia in patients with severe COVID-19 was found. In patients with severe COVID-19 course, the absolute counts of CD3<sup>+</sup>, CD4<sup>+</sup>, and CD8<sup>+</sup> T lymphocytes at admission were lower than on day 14 after discharge. Further analysis showed that the older were the patients with COVID-19, the more likely they developed severe infection. The results confirmed the significance of T lymphocytes in the clearance of the COVID-19.

**Key Words:** *COVID-19 infection; lymphopenia; sex; age; absolute count of T lymphocyte subsets*

COVID-19 infection epidemics imposes continuous threat to humans and the economy as it spreads easily and leads to serious consequences for the humanity [1,3,10]. T lymphocytes are one of the main components of adaptive immune system and are of vital importance for immune defense against viruses. Several published studies showed that COVID-19 patients at admission have reduced counts of T lymphocytes [5,9]. However, association of disease severity with changes in peripheral T lymphocyte subsets are poorly studied.

This study was intended to better define T lymphocyte subsets in different clinical groups of COVID-19 patients to explore the interaction between T cell-mediated immune response and the severity of COVID-19 infection.

## MATERIALS AND METHODS

The study involved 75 COVID-19 patients aged 21-78 years from the First Affiliated Hospital of University of Science and Technology of China were divided

into groups with severe (including severe and critical) and moderate (including mild and common) course of the infection according to the Chinese Clinical Guidance for COVID-19 Pneumonia Diagnosis and Treatment [2]. On day 14 after discharge, all patients were tested on the presence of viral RNA in throat swab by fluorescence quantitative PCR. Since all participants had negative results, their parameters of blood analysis on day 14 after discharge were taken as values of healthy controls.

This study was approved by the Ethics Committee of the First Affiliated Hospital of University of Science and Technology of China, with written informed consent obtained from each patient.

The total counts of white blood cells (WBC) and the percentages and absolute numbers of total lymphocytes were determined on a BC-5800 automated hematologic analyzer (Mindray). To examine T lymphocyte subsets, immunofluorescence was measured on a FACSCalibur (BD) flow cytometer using CellQuest software.

The results were statistically processed using GraphPad Prism 5.0 software (GraphPad Software, Inc.). The significance of differences in peripheral T lymphocyte populations at admission and on day 14 after discharge (control) was assessed using the paired

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**TABLE 1.** Blood Count Parameters in Group with Different Clinical Types of COVID-19

Group	Day 1 of admission			Day 14 of discharge		
	leukocytes	lymphocytes		leukocytes	lymphocytes	
		abs.	%		abs.	%
Moderate group (n=47)	5.83±2.36	1.56±0.89	29.18±15.03	5.52±1.37	1.56±0.50	29.13±9.27
Severe group (n=28)	6.67±3.67	0.9±0.5**	15.59±9.41**	5.86±1.52	1.35±0.38	25.19±11.84

**Note.**  $p < 0.05$  in comparison with \*day 1 after admission, †moderate group.

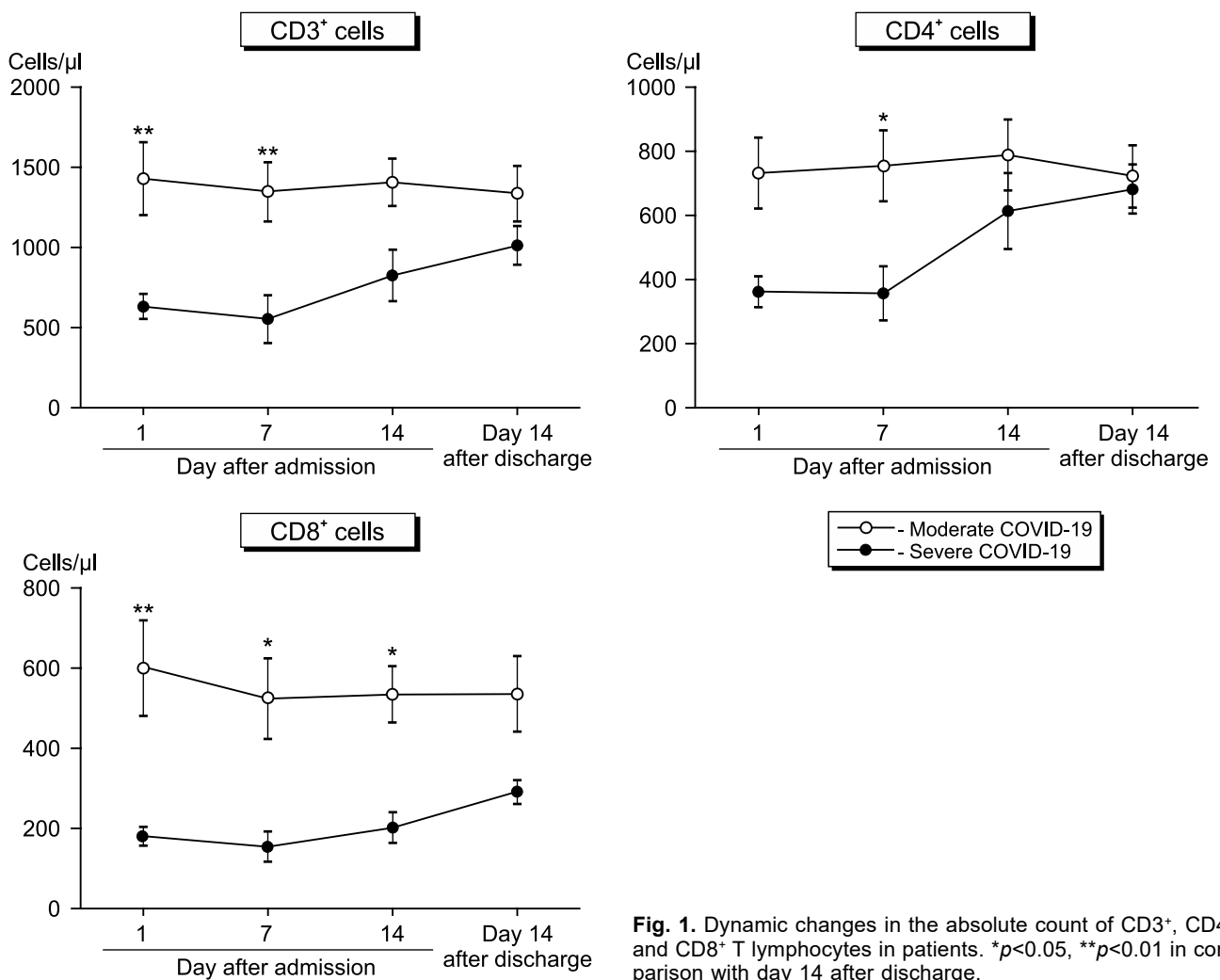
and unpaired Student's *t* test were used for comparing the groups of severe and moderate COVID-19.

## RESULTS

In 28 (34%) of 75 cases, COVID-19 run a severe course (Table 1). In the severe patients, the relative and absolute lymphocyte counts on day 1 after admission were significantly lower than in the same individuals after convalescence and then in patients with

moderate course of COVID-19. The results indicated that during the initial stage of infection, patients with severe COVID-19, rather than patients with moderate course of the disease, developed lymphopenia.

Since age had been reported as an attribution for the severity of COVID-19 infection [4,8], 75 patients were divided into three age groups. On day 1 after admission, reduced T lymphocyte counts were observed in groups aged 39-58 and 59-78 years in comparison with the corresponding values on day 14 after



**Fig. 1.** Dynamic changes in the absolute count of CD3<sup>+</sup>, CD4<sup>+</sup>, and CD8<sup>+</sup> T lymphocytes in patients. \* $p < 0.05$ , \*\* $p < 0.01$  in comparison with day 14 after discharge.

**TABLE 2.** Analysis of Different T Lymphocyte Subsets in Patients of Different Age Groups with Severe and Moderate COVID-19

Age group	Day 1 of admission				Day 14 of discharge			
	CD3 <sup>+</sup> cells	CD4 <sup>+</sup> cells	CD8 <sup>+</sup> cells	CD3 <sup>+</sup> cells	CD4 <sup>+</sup> cells	CD8 <sup>+</sup> cells	CD4 <sup>+</sup> cells	CD8 <sup>+</sup> cells
21-38 years (n=24)	2,151±1,194	1,132.0±678.1	879.1±531.9	1,646.0±356.1	913.4±275.1	634.3±207.6	913.4±275.1	634.3±207.6
moderate group (n=21)								
severe group (n=3)	671.7±219.9*	309.30±73.08*	323.3±191.8	1,442.0±400.2*	521.3±205.8	401.70±86.56*	521.3±205.8	401.70±86.56*
39-85 years (n=37)	917.8±575.6	520.4±317.3	339.5±259.3	1,127.0±449.3	662.7±228.1	402.5±220.1	662.7±228.1	402.5±220.1
moderate group (n=23)								
severe group (n=14)	635.3±238.1*	374.9±148.8*	211.30±76.59*	1,369.0±503.7	764.3±276.3	530.7±395.8	764.3±276.3	530.7±395.8
59-78 years (n=14)	935.0±635.1	545.2±328.2	294.6±326.7	1,043.0±372.9	643.6±293.5	311.2±183.7	643.6±293.5	311.2±183.7
moderate group (n=4)								
severe group (n=10)	482.3±260.9*	312.2±169.6*	145.80±86.84*	998.5±308.3	686.5±307.9	285.30±73.64	686.5±307.9	285.30±73.64

**Note.** p<0.05 in comparison with \*day 1 after admission, †moderate group.

**TABLE 3.** Sex Differences in the Absolute Count of T-Lymphocyte Subsets in Patients with Severe and Moderate COVID-19

Sex	CD3 <sup>+</sup> cells	CD4 <sup>+</sup> cells	CD8 <sup>+</sup> cells	CD3 <sup>+</sup> cells	CD4 <sup>+</sup> cells	CD8 <sup>+</sup> cells
Female (n=31)	1,219±1124	697.1±627.2	443.4±476.3	1,157.0±447.6	729.9±324.7	373.4±164.7
moderate group (n=24)						
severe group (n=7)	620.5±297.5	361.3±185.9	172.8±56.98	1,124.0±499.9	582.3±254.5	286.3±89.69
Male (n=44)	1,785.0±909.9*	912.2±466.8*	763.1±429.5*	1,634.0±437.7	873.9±221.9	684.1±268.0
moderate group (n=23)						
severe group (n=21)	564.8±225.1**	336.5±126.5**	207.20±121.4**	1,183.0±414.7	663.2±246.1	481.0±320.1

**Note.** p<0.05 in comparison with \*day 1 after admission, †moderate group.

discharge (Table 2). In the group aged 21-38 years, T lymphocyte subset counts were obviously decreased in patients with severe COVID-19 in comparison with the values observed in the moderate groups. Our results demonstrated that the decline in total lymphocyte counts was attributed to a decrease in the count of T lymphocytes, which was associated with age.

Next, we examined the composition of T lymphocyte subpopulations in peripheral blood from patients of different sex. The study included 44 male and 31 female patients. Of 44 males, severe and moderate course of COVID-19 infection was observed in 21 and 23 patients, respectively. In the female group, severe cases account for 23% (7 of 31 patients; Table 3). This suggests that male patients are easier to develop severe conditions.

Finally, dynamic changes in the balance of T lymphocyte subsets were explored. It was found that significant drop in CD3<sup>+</sup> and CD4<sup>+</sup> T lymphocyte counts persisted for at least 7 days after infection, and it could take more days for CD8<sup>+</sup> T lymphocyte to return to relatively normal level (Fig. 1). The results showed that the counts of various subsets of peripheral T lymphocytes were related to the severity and prognosis of COVID-19 infection. We therefore conclude that marked decrease in T lymphocyte subsets counts can be used to assess disease severity, and treatment effect of COVID-19 patients.

In the study alterations were intuitively assessed by comparing absolute numbers of peripheral T lymphocyte subsets between the infection group and own control group. We demonstrated that a significant decrease in the counts of peripheral T lymphocyte subsets is a unique characteristic of patients with severe COVID-19, whereas the values in patients with moderate course of the disease remain comparable with the normal. Immunosuppression in patients with severe COVID-19 was more obvious, which was consistent with the opinions of other experts [6]. Moreover, we found that males were more prone to develop severe COVID-19 than females. It was previously suggested that sex differences may be a factor in sustaining COVID-19 immunity responded to thymosin  $\alpha$ 1, male and female show significant differences in relevance to cytokine production associated with the development of a more significant number of symptoms [7].

Hence, our findings suggest that absolute numbers of peripheral T lymphocyte subsets as well as patient's age and gender should be taken into consideration when prescribing treatment to a patient who has not yet identified clinical type.

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