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COVID-19: Lymphocyte Subpopulations Monitoring in Critically Ill Patients

Amra Ziadi[1], Abdelhamid Hachimi[2*], Raja Hazime[3], Imane Brahim[3], Brahim Admou[3], Fouzia Douirek[1], Ahmed R. El Adib[4], Said Younous[4], Abdenasser M. Samkaoui[1]

[1]Polyvalent Intensive Care Unit, Arrazi Hospital, Mohammed VI[th] University Centre, Cadi Ayyad University, Marrakech, Morocco
[2]Medical Intensive Care Unit, Arrazi Hospital, Mohammed VI[th] University Centre, Cadi Ayyad University, Marrakech, Morocco
[3]Laboratory of Immunology, Centre of Clinical Research, Mohammed VI[th] University Centre, Cadi Ayyad University, Marrakech, Morocco
[4]Polyvalent Intensive Care Unit, Child and Mother Hospital, Mohammed VI[th] University Centre, Cadi Ayyad University, Marrakech, Morocco
Email: *abdelhachimi@gmail.com

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Abstract

Background: The alteration of lymphocyte subpopulations can help to predict the severity and the prognosis of severe Coronavirus disease 2019 (COVID-19). Our goal was to describe the kinetics of lymphocyte subsets, and their impact on the severity and mortality in critically ill COVID-19 patients. **Methods:** We collected demographic data, comorbidities, clinical signs on admission, laboratory findings on admission then a follow-up during hospitalization. Lymphocyte subsets including CD3+ T cells, CD4+ T cells, CD8+ T cells, B cells, and natural killer (NK) cells were counted by flow cytometer. **Results:** On admission, we observed lymphopenia in 57% of cases, decreased CD3+ T cells in 76% of cases, decreased CD4+ T cells in 81% of cases, decreased CD8+ T cells in 62% of cases, decreased B cells in 52% of cases, and decreased natural killer (NK) cells in 33% of cases. After treatment, decreased CD3+ T cells, decreased CD4+ T cells, decreased CD8+ T cells, and decreased natural killer cells were predictor factors of mortality, in the univariable analysis. **Conclusion:** CD3+ T cells, CD4+ T cells, CD8+ T cells, and natural killer cells were predictor factors of severity, ICU mortality, and also a useful tool for predicting disease progression.

Keywords

SARS-CoV-2, Coronavirus Disease 2019, Lymphocyte Subsets, Critical Care Outcomes

1. Introduction

In December 2019, a new disease due to SARS-CoV-2 infection appeared in China. A few weeks later, it spreads and becomes a pandemic; about 6,931,000 cases have been infected globally with 400,857 (5.8%) deaths, as of June 8, 2020 [1]. The pathophysiology of this infection remains not fully clarified. Although, it has been observed that SARS-Cov-2 is responsible for a reactive inflammatory storm as a result of an exaggerated host immune system response, with deleterious effects on multiple organs; firstly, in the most of the cases, it affects pulmonary tract, then, the others systems including cardiovascular, gastrointestinal, neurological, hematopoietic and immune system [2] [3] [4].

Even though T lymphocytes and natural killer cells are essential to control viral infections, lymphopenia is a frequent hematological disorder in serious COVID-19 patients [5] [6] [7]. This abnormality might be explained by firstly, the virus may directly affect lymphocytes [8], because of the expression of angiotensin-converting enzyme-2 on the surface of lymphocytes [9] [10]. Secondly, their inhibition by metabolic disorders (*i.e.* lactic acidosis) [11]. Thirdly, pro-inflammatory cytokines including tumor necrosis factor (TNF)α, and interleukin (IL)-6, could provoke lymphocyte deficit, and fourthly, the virus might engender lymphatic organs damage such as thymus and spleen [12]. Thus, the occurrence rate was between 44.5% [13], 67% [14], and 92.6%, (15) of critically ill COVID-19 cases, because of they adopted different definitions: lymphocytes < 500/mm^3 [13], lymphocytes < 1000 mm^3 [14], or lymphocytes < 1500 mm^3 [15]. Moreover, lymphocyte subpopulations decreased and predicted the severity and the prognosis of severe COVID-19 [16] [17]. Herein, our goal was to describe the kinetics of lymphocyte subsets, and their impact on the severity and mortality in critically ill COVID-19 patients.

2. Methods

For this prospective single-center study, we included all adult patients with confirmed COVID-19 infection by a positive reverse-transcriptase-polymerase-chain-reaction (RT-PCR) assay of a nasopharyngeal swab, admitted in the intensive care unit (ICU) of the Mohammed VIth University Centre of the Marrakech region, Morocco, from Mach 19, 2020, to May 15, 2020.

Critically ill patients were defined as admitted in the ICU because they required mechanical ventilation or more than eight liters per minute of oxygen to maintain pulse oxygen saturation (SpO_2) > 90% or had a respiratory rate of more than 40 breaths per minute.

We collected demographic data, clinical signs, laboratory findings on admission then a follow-up during hospitalization (lymphocytes, D-dimer, ferritin, lactate dehydrogenase (LDH), C-reactive protein, procalcitonin, PaO_2:FiO_2), chest CT scan if available, outcomes, time from onset of the first symptom to ICU admission, Charlson Comorbidity Index [18] and sequential organ failure assess-

ment (SOFA) scores [19]. CD3+ T cells, CD4+ T cells, CD8+ T cells, B cells, and natural killer (NK) cells were counted by flow cytometer. All tests were performed at the discretion of the treating physician.

We expressed continuous variables as medians and interquartile (IQR) ranges or means (standard deviations (SD)), as appropriate. Categorical variables were described using percentages and compared using the χ^2 test, although Fisher exact test was used when the data were sparse. We performed a univariable analysis to evaluate the risk factors of mortality. The analysis was processed by SPSS 10.0 for Windows (SPSS, Chicago, IL, USA). A p-value of less than 0.05 was considered statistically significant.

Informed consent was waived due to the emergency of the disease, and researchers analyzed only anonymized data. All research was conducted following the national guidelines and regulations.

3. Results

Of 1618 COVID-19 patients hospitalized in our university center, 55 patients (3.4%) were admitted to the ICU. We list the basic, clinical characteristics, biological and radiological findings in **Table 1**. The mean age was 59 (16.5) years; 74.5% were men. Among all the patients, 84% had chronic medical conditions. The frequent symptoms were dyspnoea (85%), and cough (80%). The median length from the onset of symptoms to ICU admission was 7 (6 - 8) days. The median SOFA score on admission was 5 (4 - 17). On admission, lymphopenia was common (76%) with a median of 980/mm^3, the median LDH was 560 IU/L, the median D-dimer was 2975 mg/L, the median ferritin was 1135 ng/mL. The chest CT scan showed bilateral ground-glass opacification > 50% in 74% of cases.

Among the 55 patients, we analyzed the lymphocyte subsets from 21 patients. On admission, we observed lymphopenia in 57% of cases, decreased CD3+ T cells in 76% of cases, decreased CD4+ T cells in 81% of cases, decreased CD8+ T cells in 62% of cases, decreased B cells in 52% of cases, and decreased natural killer (NK) cells in 33% of cases. In these 21 patients, 71.4% (15/21) received chloroquine/hydroxychloroquine plus azithromycin, and 4.8% (1/21) treated with lopinavir/ritonavir. The monitoring of the lymphocyte subpopulation counts was reported in **Figure 1**. Effectively, after treatment, only 2/9 non-survivor patients improved their CD3+ T cells versus 10/12 survivor patients. Besides, regarding the CD4+ T cells, 11/12 survivor patients presented a normal count versus 1/9 non-survivor patients. The mortality rate was 43.6% of cases (24 patients). On admission, none of the decreased lymphocyte subsets was associated with mortality. However, after treatment, decreased CD3+ T cells (78% vs 17%; $p = 0.009$), decreased CD4+ T cells (90% vs 8%; $p < 0.001$), decreased CD8+ T cells (90% vs 8%; $p < 0.001$), and decreased natural killer cells (67% vs 0%; $p = 0.002$) were predictor factors of mortality, in the univariable analysis (**Table 2**).

Table 1. Basic and clinical characteristics, laboratory data and chest CT scan findings of all patients.

Characteristics	All patients (N = 55)
Mean age (SD) (year)	59 (16.5)
Sex (%)	
Male	74.5
Female	25.5
Comorbidities (%)	84
Hypertension	42
Diabetes	34
Coronary heart disease	11
Chronic kidney disease	9
Chronic obstructive pulmonary disease	4
Cerebrovascular disease	4
Cancer	4
Asthma	2
Cirrhosis	2
Connective tissue disease	2
Smoking	16
Alcoholism	4
Others	11
Charlson Comorbidity Index score, median (IQR)	3 (2 - 5)
Length from the onset of symptoms to ICU admission, median (IQR) (day)	7 (6 - 8)
Symptoms (%)	
Dyspnea	85
Cough	80
Respiratory struggle	54
Fever	26
Digestive signs	26
Agitation	22
SOFA score	
On admission, median (IQR)	5 (4 - 17)
Highest score during the first three days, median (IQR)	10 (5 - 16.5)
On day 7, median (IQR)	11 (4 - 16.75)

Continued

Laboratory data	
On admission	
Lymphocytes count, median (IQR) (per mm^3)	980 (565 - 1455)
D-dimer, median (IQR) (mg/L)	2975 (1490 - 7112)
Ferritin, median (IQR) (ng/mL)	1135 (547 - 2023)
LDH, median (IQR) (IU/L)	560 (381 - 766)
C-reactive protein, median (IQR) (mg/L)	173 (99 - 243)
Procalcitonin, median (IQR) (ng/mL)	0.31 (0.13 - 0.71)
PaO_2:FiO_2 ratio, median (IQR)	86 (70 - 130)
During the first 3 days	
Lowest lymphocytes count, median (IQR) (per mm^3)	810 (655 - 1035)
Highest D-dimer level, median (IQR) (mg/L)	3225 (289 - 9992)
Highest ferritin level, median (IQR) (ng/mL)	1416 (688 - 1940)
Highest LDH level, median (IQR) (IU/L)	560 (381 - 766)
Highest C-reactive protein level, median (IQR) (mg/L)	175 (111 - 253)
Highest procalcitonin level, median (IQR) (ng/mL)	2 (0.38 - 3.85)
Lowest PaO_2:FiO_2 ratio, median (IQR)	86 (70 - 130)
On day 7	
Lymphocytes count, median (IQR) (per mm^3)	763 (570 - 1540)
D-dimer level, median (IQR) (mg/L)	4045 (2752 - 9877)
Ferritin level, median (IQR) (ng/mL)	1325 (734 - 1940)
LDH, median (IQR) (IU/L)	402 (271 - 507)
C-reactive protein level, median (IQR) (mg/L)	98 (52 - 170)
Procalcitonin level, median (IQR) (ng/mL)	0,59 (0.18 - 4.25)
PaO_2:FiO_2 ratio, median (IQR)	98 (78 - 148)
Chest CT scan (%)	
Ground glass opacification <25%	10
Ground glass opacification 25% - 49%	16
Ground glass opacification 50% - 74%	37
Ground glass opacification 75% - 100%	37
Pleural effusion	0

IQR, interquartile range. SOFA, sequential organ failure assessment. LDH, lactate dehydrogenase. PaO_2:FiO_2, the ratio of the partial pressure of arterial oxygen to the fraction of inspired oxygen.

Table 2. Predictor factors of mortality in univariable analysis.

	Before treatment			After treatment		
	Non-survivors	Survivors	p	Non-survivors	Survivors	p
Decreased CD3+ T cells (%)	89	68	0.3	78	17	0.009
Decreased CD4+ T cells (%)	88	75	0.6	90	8	<0.001
Decreased CD8+ T cells (%)	88	42	0.06	90	8	<0.001
Decreased B cells (%)	56	50	0.9	11	0	0.4
Decreased natural killer cells (%)	34	33	0.9	67	0	0.002

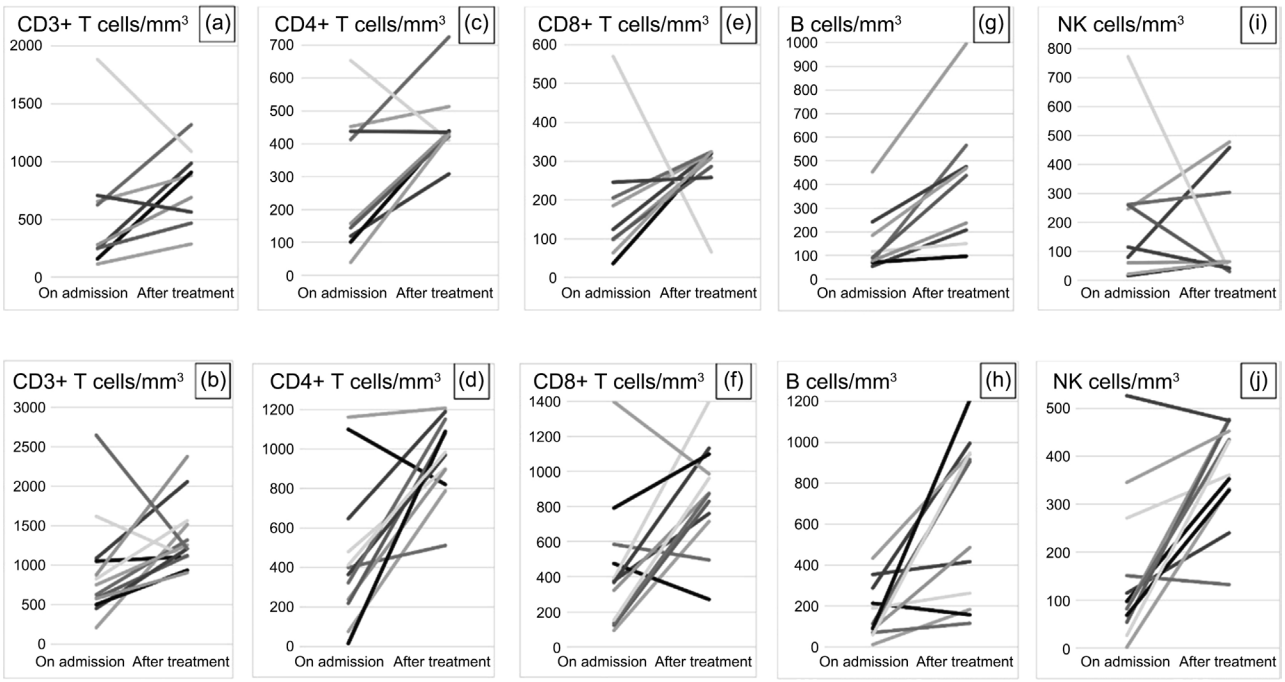

Figure 1. The kinetics of lymphocyte subpopulations (CD3+ T cells, CD4+ T cells, CD8+ T cells, B lymphocytes, and natural killer (NK)) between admission and after treatment in non-survivor patients ((a), (c), (e), (g), and (i)) and in survivor patients ((b), (d), (f), (h), and (j)).

4. Discussion

In our population, after treatment, decreased CD3+ T cells, decreased CD4+ T cells, decreased CD8+ T cells, and decreased natural killer cells were predictor factors of mortality; further, a large proportion of patients recovered lymphocyte subpopulations in survivor cases. At the best of our knowledge, this is the first report of the monitoring lymphocyte subsets in a Moroccan cohort of critically ill COVID-19 patients.

These findings were in line with the publishing data. Wang *et al.* [20] declared the count alteration of total lymphocytes, CD4+ T cells, CD8+ T cells, B cells, and natural killer cells in COVID-19 patients; as well, the CD8+ cells was an independent marker of severity and efficacy of treatment. And accordingly to Sun *et al.* [21] who observed that total T lymphocytes, CD4+ T lymphocytes, CD8+ T

lymphocytes, and NK cells decreased in un-discharged/died group at two weeks after treatment, compared with the discharged group. Additionally, CD4+ T cells and CD8+ T cells could help to evaluate the disease evolvement [22]. Besides, a recent meta-analysis concluded that lymphopenia was related to worsened outcomes [23].

This study has some limitations. The first concern was that our study was a single-centered study with only 55 severe patients, of whom only 21 patients who had lymphocytes subset counts. Secondly, we collected lymphocyte subset counts on admission in the intensive care unit, but some patients were initially hospitalized in a general ward for some days. Thirdly, the interpretation of our findings might be limited by the sample size. However, our ICU was the referral center in the region, thus we consider our study population is representative of cases diagnosed and treated in our region. Further studies through the country still needed.

5. Conclusion

CD3+ T cells, CD4+ T cells, CD8+ T cells, and natural killer cells were predictor factors of severity and ICU mortality. As well, they were a useful tool for predicting disease progression. Other larger sample studies are needed to validate risk factors and the lymphocyte threshold.

Ethical Considerations

Informed consent was waived due to the emergency of the disease, and researchers analyzed only anonymized data. All research was conducted following the national guidelines and regulations.

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Authors' Contributions

A.Z., A.H., H.R., B.A., and A.M.S. designed the study; A.Z. and A.H. wrote and edited the paper; R.H., I.B., B.A., and A.R.E. discussed results, and edited the paper; F.D., Y.Z., H.C., and S.Y. discussed results and edited the paper.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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