CASE REPORT

T-cell immunophenotyping in COVID-19 pneumonia

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The COVID-19 epidemic is currently a global threat that has affected many parts of the world. Some patients require intensive care unit admission due to severe symptoms in the course of the disease. The severity of symptoms in this disease varies from person to person. The effectiveness of the immune response against viral infections depends on the number and activity of T-cells, which play an important role in eliminating virus-infected cells. In this study, we report two patients with COVID-19 pneumonia, one with moderate symptoms and the other with severe symptoms. Although a decrease of absolute lymphocyte count was seen in both patients, a more significant decline reported in the ICU-admitted patient. Expression of activated markers, HLA-DR, CD38, on CD8-positive T-cells was shown in a patient with more severe disease. On the other hand, partial loss of CD7 in the severe case was also observed. Hence, besides of the above parameters that already mentioned in other studies, loss of pan T-markers could be considered as a potentially valuable test for predicting disease severity. We suggest evaluating the predictability of these tests in COVID-19 in larger studies. This study was approved by the Ethics Committee of Isfahan University of Medical Sciences (IR.MUI.MED.REC.1399.238).

Key words: COVID-19, flowcytometry, T cell immunophenotyping

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INTRODUCTION

Coronaviruses include a large group of respiratory viruses that can manifest as a simple cold to middle-east respiratory syndrome and severe acute respiratory syndrome.[1,2] In December 2019, the COVID-19 epidemic occurred in Wuhan, Hubei Province, China, which is now considered a global threat.[3] Clinical manifestations include fever, cough, muscle aches, fatigue, diarrhea, pneumonia, and in severe cases, death.[4-6] The severity of symptoms in this disease varies from person to person. The severe disease sometimes manifests as acute respiratory distress syndrome and require intensive care unit (ICU) admission.[4] The effectiveness of the immune response against viral infections depends on the number and activity of T-cells, which play an important role in eliminating virus-infected cells.[7] CD8 lymphocytes play a key role in the treatment of acute viral pneumonia.[8] Studies have shown that many patients with COVID-19 have lymphopenia.[4] The absolute number of CD4 and CD8 T cells in patients with COVID-19 decreased significantly compared to the control group.[9] It was also stated that, compared to hospitalized patients in the ICU and patients without the need for an ICU, CD4 T-cells decreased in both groups, although CD8 T-cells had a more significant decrease in patients in need of ICU.[10] If clinical and paraclinical variables can be used to predict the course of the disease, it is possible to prevent severe symptoms with preventive interventions. It seems that evaluating the number of T-cells and markers expressed at the level of these cells can be a criterion for predicting disease progression. This paper describes the clinical status of two patients

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with COVID-19 infection and their peripheral blood flow cytometry findings.

CASE REPORTS

Case 1

A 52-year-old female presented to the emergency department with a history of cough and dyspnea on exertion from 1 week ago. She also mentioned dry cough, diarrhea, and myalgia in the past 3 days. Due to the progression of dyspnea and history of contact with a COVID-19 patient, she was admitted to the hospital. Hypertension was her only underlying disease and treated with metoprolol 50 mg daily. In physical examination except for hypoxia, no other abnormalities were detected. She had an O_2 saturation of 89% in the air room.

COVID-19 reverse transcription polymerase chain reaction (RT-PCR) was reported positive and other laboratory tests are shown in Table 1. A bilateral ground-glass pattern was seen in chest high-resolution computed tomography (HRCT).

After her admission, she underwent conservative treatment, antibiotic, and hydroxychloroquine (200 mg every 12 h for 5 days). Seven days later, hypoxia and general condition improved and she was discharged from the hospital.

Table 1: Laboratory tests

Variable	Reference range	Case 1	Case 2
WBC (per mm^3)	4400-11,000	5600	5200
Differential count (%)			
Lymphocytes	20-40	50	26
Neutrophils	50-70	48	72
Hemoglobin (g/dl)	14-16.5	12.6	11
Platelet (per mm^3)	150,000-450,000	426,000	168,000
BUN (mg/dl)	16-25	17.7	19
Cr (mg/dl)	0.5-1.5	0.92	0.7
AST (units/L)	<30	27	29
ALT (units/L)	<30	26	28
ALP (units/L)	50-350	126	130
Na (mEq/L)	135-145	137	128
K (mEq/L)	3.5-5	4.1	4.2
Ca (mg/dl)	8.5-10.5	8.8	8.7
Ph (mg/dl)	2-4	3.5	4.3
Alb (g/dl)	4-5	3.8	2.55
CRP (mg/L)	up to 5	4	27
LDH (U/L)	Up to 450	356	1084
INR	1	1	1
PH	7.35-7.45	7.42	7.45
pCO_2 (mmHg)	35-45	50	53.4
HCO_3 (mEq/L)	22-24	25	23

BUN=Blood urea nitrogen; Cr=Creatinine; AST=Aspartate aminotransferase; ALT=Alanine aminotransferase; ALP=Alkaline phosphatase; Na=Sodium; K=Potassium; Ca=Calcium; Ph=Phosphorus; Alb=Albumin; CRP=C reactive protein; LDH=Lactate dehydrogenase; pCO_2=Partial pressure of carbon dioxide; HCO_3=Bicarbonate; WBC=White blood cells; INR=international normalized ratio

Case 2

A 36-year-old female was admitted to the emergency department with fever and cough. Her respiratory symptoms started 4 days earlier and gradually deteriorated. She had hypothyroidism, a history of surgery for a benign brain tumor, and seizure 4 years ago. Drug history was depakene, levetiracetam, levothyroxine, and lacosamide.

At admission, she had tachypnea (Respiratory rate: 28/min), tachycardia (Pulse rate: 110/min), fever (T: 39°C), and severe hypoxia (O_2 saturation of 79% in air room). Other examinations were normal.

COVID-19 RT-PCR was reported positive, and other laboratory tests are shown in Table 1. Chest HRCT findings were bilateral ground-gloss pattern and patchy alveolar infiltration.

Due to severe hypoxia, she was admitted to the ICU. Conservative treatment, antibiotics, hydroxychloroquine (200 mg every 12 h for 10 days), favipiravir, interferon β, and dexamethasone (8 mg IV every 12 h) were prescribed for the patient during ICU care.

Due to her obesity, hypoxia, and hypoventilation, BIPAP was started for the patient, and after 15 days, hypoxia and general condition were improved, and she was discharged with BIPAP from the hospital.

For both patients, peripheral blood flowcytometry was performed as following:

Whole blood samples were collected in the EDTA tubes and were submitted to flowcytometry laboratory for immunophenotyping of lymphoid cells by multicolor flowcytometry analysis. Partrc flow cytometry (Germany) was employed, and results analysis was performed by FloMax software. The following antibodies in three tubes were used, and preparation was performed according to the manufacturer's instructions:

1. Anti-CD20 (PerCP)/anti-CD5 (FITC)/anti-CD7 (PE)
2. Anti-CD4 (PE)/anti-CD8 (FITC)/anti-CD3 (PerCP)
3. Anti-CD8 (FITC)/anti-CD38 (PE)/HLA-DR (PreCP).

All antibodies and other solutions were purchased from Exbio, CZ/SK Company, Germany. At least 30,000 events were acquired and analyzed. Small and large lymphoid cells were gated based on forward and side scatter plots, and expression of variable antigens was evaluated. B and T lymphocytes based on CD20 and CD3 expression were counted. Expressions of all pan T markers (CD2, CD3, CD5, and CD7) accompany with CD4 and CD8, and expression of activated markers of T lymphocytes including HLA-DR

Table 2: Peripheral blood flowcytometry results

	Case 1	Case 2
Total lymphocyte count (/μL)	1900	1240
B lymphocyte (/μL)	285	170
T lymphocyte (/μL)	1270	690
CD4+ T lymphocyte (/μL)	800	320
CD8+ T lymphocyte (/μL)	380	280
Expression of HLA-DR on T cells	No	Yes
Expression of CD38 on T cells	No	Yes
Partial CD7 loss	No	Yes

and CD38 also was analyzed. Expression of aberrant HLA-DR and CD38 and CD7 loss was seen in severe case [Table 2].

DISCUSSION

Lymphocyte count including T-cells and B-cells was reduced in COVID-19 infection, especially in elderly ones and in patients who need ICU care. Non-ICU patients with decreased total, CD4, and CD8-positive counts need close intervention even in the absence of severe symptoms due to further risk for disease progression.[10] Aberrant T cell phenotype (including pan T cell antigen loss) is not specific for T-cell neoplasms, different reactive processes may cause different T-cell subpopulations with phenotype changes. Partial or complete loss of one or even two T-cell markers may be observed in reactive processes including in viral infection. The antigens most commonly showing aberrant expression are CD7, followed by CD5. Samples from some viral diseases such as infectious mononucleosis may exhibit activated markers (HLA-DR and CD38).[11] Increased activation markers (HLA-DR and CD38) on CD8 positive T-cells are more frequent in COVID-19 infection which required hospitalization than the normal population.[12] In our report, case 2 who presented with severe symptoms and needed ICU care showed a lower number of all lymphoid subgroups, partial loss of CD7, and expression of activated markers on T cells in comparison to case 1 who did not need ICU care.

CONCLUSION

Each of the above parameters that already mentioned in other studies as well as the loss of pan T-markers which according to our search was not introduced in other studies could be considered as a potentially valuable test for predicting disease severity and we suggest evaluating the predictability of these tests in COVID-19 in larger studies.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Conflicts of interest

There are no conflicts of interest.

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