Original Article

Iranian Journal of Immunology

March 2025; 22(1):70-82.

Evaluation of Dendritic Cell Subpopulations Frequency in COVID-19 Patients and their Correlation with Disease Severity

Vahid Aghariazar[1#], Majid Eterafi[2#], Somaieh Matin[3], Nasrin Fouladi[4, 5], Rozita Abolhasani[1], Monireh Falsafi[1], Afshin Fathi[6*], Elham Safarzadeh[1, 7*]

[1]Cancer Immunology and Immunotherapy Research Center, Ardabil University of Medical Sciences, Ardabil, Iran; [2] Students Research Committee, Ardabil University of Medical Sciences, Ardabil, Iran; [3]Department of Internal Medicine, Emam Khomeini Hospital, Ardabil University of Medical Sciences, Ardabil, Iran; [4]School of Medicine and Allied Medical Sciences, Ardabil University of Medical Sciences, Ardabil, Iran; [5]Social Determinants of Health Research Center, Ardabil University of Medical Sciences, Ardabil, Iran; [6]Pediatric Hematology and Oncology Department, Ardabil University of Medical Science, Ardabil, Iran; [7]Department of Microbiology, Parasitology, and Immunology, Ardabil University of Medical Sciences, Ardabil, Iran
# These two authors contributed equally to this work.

ABSTRACT

Background: COVID-19 (2019) clearly demonstrates an imbalanced immune response. Variations in the function and subtypes of dendritic cells (DCs) may have effects on immune responses in COVID-19 patients and contribute to immunopathology.

Objectives: To assess the phenotype and frequency of Plasmacytoid dendritic cells (pDCs), Conventional DCs (cDCs), and double-positive DCs in COVID-19 patients admitted to the ICU and non-ICU compared to the healthy control group.

Methods: The study included 10 healthy individuals and 25 COVID-19 patients. In the second week of their illness, Peripheral blood mononuclear cells (PBMCs) were isolated from the patients and labeled with targeted antibodies for HLA-DR, CD123, and CD11c. The samples were then analyzed using flow cytometry. The COVID-19 patients were divided into two ICU and non-ICU groups and were closely monitored throughout the study.

Results: In comparison to healthy controls, COVID-19 patients exhibited a significantly lower pDCs ratio (P=0.04). Patients were categorized into two groups: (A) the ICU group (n=11; 44%) and (B) the non-ICU group (n=14; 56%). The frequency of pDC was significantly lower in ICU patients than in non-ICU patients (P<0.01). Although not statistically significant, ICU patients had a lower frequency of cDCs and double positive DCs compared to non-ICU patients. Additionally, a significant association between the age of COVID-19 patients and cDC levels was observed (P=0.049).

Conclusion: SARS-CoV-2 can evade attacks from the immune response by reducing the number of DCs and suppressing their function of DCs, ultimately resulting in weakened development of both innate and adaptive immunity.

Keywords: CD11c; CD123; Corona virus; Dendritic cells; HLA- DR

*Corresponding author:
Elham Safarzadeh,
Department of Microbiology, Parasitology and Immunology, School of Medicine, Ardabil University of Medical Sciences, Ardabil, Iran
Email: Elham.im63@gmail.com
Afshin Fathi,
Department of Pediatric Oncology, Ardabil University of Medical Sciences, Ardabil, Iran
Email: a.fathi@arums.ac.com

Cite this article as:
Asghariazar V, Eterafi M, Matin S, Fouladi N, Abolhasani R, Falsafi M, Fathi A, Safarzadeh E. Evaluation of Dendritic Cell Subpopulations Frequency in COVID-19 Patients and their Correlation with Disease Severity. *Iran J Immunol.* 2025; 22(1):70-82, doi: 10.22034/iji.2025.104236.2887.

Received: 2024-09-24
Revised: 2024-12-17
Accepted: 2025-01-06

INTRODUCTION

Dendritic cells (DCs) are an broad category of antigen-presenting cells (APCs) that includes different subsets such as $CD123^+$ plasmacytoid DCs (pDC) and conventional (cDC) $CD1c^+$ and $CD141^+$ DCs (1). These innate cells play crucial roles in immunological functions, including initiating, regulating, and maintaining both innate and acquired responses, such as activating of adaptive $CD4^+$ and $CD8^+$ T cell responses. Additionally, they are essential in the immune responses to viral and bacterial infections (2). Conventional DCs play a pivotal role in eliminating viral infection and are particularly skilled at presenting antigens that activate and expand T cells, bridging the gap between specific and non-specific immune responses (3, 4). In contrast, pDCs have limited ability to promote T cells, but they excel at generating abundant type I interferon (IFN) and mediating antiviral responses (5). Studies have shown that defects in IFN-I immunity can contribute to the severity of viral infections (6). Moreover, DCs are found circulating in blood and lining the epithelial surface of the respiratory tract where they serve as primary sensors and responders to potential threats (7). The recruitment of DCs to the respiratory tract has been observed in various human respiratory viral infections including influenza virus, hantavirus infection, and respiratory syncytial virus (RSV) (7). Changes in the homeostasis of specific populations of DCs have been associated with chronic inflammation (8). Therefore, it is essential to thoroughly evaluate myeloid cell populations in viral infections to gain new insights that may aid in the development of effective targeted treatments.

The new coronavirus disease 2019 (COVID-19) belongs to the enveloped RNA virus class and has a crown-like appearance with a diameter of about 60–140 nm (9, 10). The virus is transmitted through respiratory droplets from person to person when coughing, sneezing, or through close contact (11). In infected patients, the comination of acute respiratory distress syndrome and hemophagocytic lymphohistiocytosis (HLH) with cytokine storm features, leads to disease severity and increased mortality (12). Patients with coronavirus exhibit immune dysfunction such as abnormalities in lymphocyte activation, lymphopenia, elevated levels of immunoglobulin G (IgG) and total antibodies, secondary bacterial infections, as well as myocardial and kidney damage (13). It is crucial to understand the complex mechanisms at the cellular and molecular levels that regulate the immune response to COVID-19 infection in order to develop effective therapies. With this goal in mind, we conducted a study to examine the alteration in in the myeloid population in the peripheral blood of patients and their correlation with the severity of COVID-19. Our study focused on exmining the prevalence of circulating conventional, plasmacytoid, and double-positive DCs in individuals with COVID-19 comparing them to a healthy control group. Additionally, we analyzed the distribution profiles of DC subsets in patients admitted to the intensive care unit (ICU) versus those who were not admitted.

MATERIALS AND METHODS

Study Design

Our research examined 25 individuals with COVID-19 who were referred to the Clinic of Ardabil University of Medical Sciences between January 2021 and March 2021. The inclusion criteria included cases diagnosed with COVID-19 according to international diagnostic standards, confirmed through laboratory tests, CT scans, and clinical examinations. Laboratory assessments, such as CBC, ESR, CRP, and Real-Time PCR tests, were administered following a confirmed diagnosis of COVID-19 based on clinical symptoms. The study did not include individuals with a medical history of consuming immunosuppressive drugs or encountering acute infections.

The control group comprised ten healthy participants, selected based on age and gender, who tested negative for COVID-19. Before participating, all patients were thoroughly informed about the analysis's objectives and provided documented consent.

Clinical Demonstrations and Laboratory Measurement

The clinical presentation of COVID-19 patients, including cardiac, respiratory, neurological, digestive, and systemic clinical signs was evaluated. The percentage of vital signs in hospitalized COVID-19 patients was measured and classified as normal or abnormal and evaluated between ICU (intensive care unit) and non-ICU groups. (Supplementary Table 1). Peripheral blood samples were taken, and various parameters were measured using the AU5800 Clinical Chemistry Analyzer (Beckman Coulter, USA), including mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), white blood cell (WBC) count, red blood cell (RBC) count, hematocrit (HCT), prothrombin time (PT), platelet (PLT), glutamic pyruvic transaminase (SGPT), glutamic oxalacetic transaminase (SGOT), international normalized ratio (INR), partial thromboplastin time (PTT), blood sugar, creatinine, urea, creatine phosphokinase (CPK) and lactate dehydrogenase (LDH). Immunoturbidimetric assays measured the C-reactive protein (CRP), and the erythrocyte sedimentation rate (ESR) was calculated using a VES-MATIC Analyser.

Blood Samples and PBMCs Isolation

During the second week of illness, the COVID-19 and both control groups had peripheral blood drawn. A 5 ml blood sample was heparinized. PBMCs were then isolated through density-gradient centrifugation (20 min, 800 ×g) using the ficoll separation technique (Baharafshan, Iran). The isolated PBMCs Were washed twice with Phosphate-buffered saline (PBS) for 7 min at 300 ×g (Bioidea, Iran) before being used for Flow cytometry assessments. The collected plasma samples were stored at -80C until analysis.

Flow Cytometry Technique

Freshly isolated PBMCs were analyzed for the number of pDCs and cDCs using flow cytometry with a FACSCalibur flow cytometer from BD Biosciences (USA). FlowJo software was utilized to analyze the data (Version 10.8.0; Becton Dickinson, Mountain View, CA). To detect DCs, labeled monoclonal antibodies were added to 1 x 10^6 PBMCs, followed by a 15 to 30 minute incubation at room temperature in the dark. We used phycoerythrin (PE)- labeled anti-human CD123, FITC-labeled anti-human HLA-DR, and PE/Cy5-conjugated anti-human CD11c (BioLegend, San Diego, CA, USA) monoclonal antibodies for DC assessment. In order to conduct the analysis, we acquired at least 10,000 live events per sample. The gating strategy involved first gating the lymphocytes and monocytes based on forward and side scatters. Then, the gated area was analyzed for its HLA-DR^+ cells. HLA-DR^+CD123^- $CD11c^+$, HLA-$DR^+CD123^+CD11c^-$, and HLA-$DR^+CD123^+CD11c^+$ cells were identified respectively as cDCs, pDCs, and double positive DCs.

Statistical Analysis

Differences between independent groups were compared using either the nonparametric Mann-Whitney test or the independent sample t-test. Additionally, a two-way ANCOVA covariance analysis was conducted to compare the mean differences of variables between groups. The Kolmogorov-Smirnov test was employed to determine the normality of quantifiable data. The data was analyzed using IBM SPSS Statistics software version 26 (IBM Corp., Armonk, NY, USA) utilizing descriptive and analytical statistics. The mean±standard deviation (SD) was used to express all data and frequency (percentage) for quantitative variables. A P-value<0.05 was considered statistically significant.

RESULTS

The Clinical Characteristics and Demographics of the Study Subjects

The study analyzed various groups' demographic characteristics, including ICU admissions, non-ICU admissions, and a control group. Additionally, factors such as age, sex, marital status, blood type, and underlying diseases were also examined (as shown in Table 1). Patients were divided into two groups: (1) the ICU group (n=11; 44%) and (2) the non-ICU group (n=14; 56%). According to our data, the majority (84%) of the study participants were over 50. In terms of sex and marrital status, on average, 48% of the participants were male, and 52% were married. Additionally, 72% of the participants had underlying diseases or post-medical histories, such as cardiovascular disease (4%), hypertension (48%), diabetes mellitus (24%), Flegel disease (4%), chronic obstructive pulmonary disease (8%) and cerebral vascular accident (16%). The study also examined the clinical presentations of COVID-19 patients, including cardiac and respiratory signs, neurology, digestive, and systemic clinical signs. Notably, shortness of breath was the most common (88%) cardiac and respiratory clinical sign, while fever (32%) and cough (56%) were common systemic clinical signs (as shown in Table 2).

The Analysis of Circulating DC Subsets in the Peripheral Blood of Healthy Controls and COVID-19 Patients

Flow cytometry analyzed the proportions and characteristics of DCs in the peripheral blood of both the case and control groups. Forward and side scatter properties were utilized to distinguish lymphocytes and monocytes, and the expression of CD11c and CD123 was further examined for HLA-DR$^+$ cells. In both groups, three main subsets of DCs were identified: CD123$^-$CD11c$^+$ cDCs, CD123$^+$CD11c$^-$ pDCs, and CD123$^+$CD11c$^+$ double positive DCs (as illustrated in Fig. 1).

Table 1. Demographical characteristics of study subjects

Variable	Category	Case N (%)	Grouped with ICU admission N (%)	Grouped with Non ICU admission N (%)	Control N (%)
Age	≤50	4 (16)	3 (27.3)	1 (7.1)	7 (46.7)
	>50	21 (84.0)	8 (72.7)	13 (92.9)	8 (53.3)
Sex	Male	12 (48.0)	5 (45.5)	7 (50.0)	10 (66.7)
	Female	13 (52.0)	6 (54.5)	7 (50.0)	5 (33.3)
Marriage	Married	13 (52.0)	6 (54.5)	7 (50.0)	7 (46.7)
	Single	12 (48.0)	5 (45.5)	7 (50.0)	8 (53.3)
Blood type	A	7 (28.0)	5 (45.5)	2 (14.3)	7 (46.7)
	B	9 (36.0)	3 (27.3)	6 (42.9)	3 (20.0)
	AB	4 (16.0)	1 (9.1)	3 (21.4)	2 (13.3)
	O	5 (20.0)	2 (18.2)	3 (21.4)	3 (20.0)
Underlying diseases Or Post medical history	Yes	18 (72.0)	6 (54.5)	12 (85.7)	6 (40.0)
	No	7 (28.0)	5 (45.5)	2 (14.3)	9 (60.0)
Underlying diseases	CVD	1 (4.0)	1 (9.1)	0 (0.0)	2 (13.3)
	HTN	12 (48.0)	4 (36.4)	8 (57.1)	3 (20.0)
	DM	6 (24.0)	3 (27.3)	3 (21.4)	4 (26.7)
	HLP	1 (4.0)	1 (9.1)	0 (0.0)	2 (13.3)
	COPD	2 (8.0)	0 (0.0)	2 (14.3)	1 (6.7)
	CVA	4 (16.0)	2 (18.2)	2 (14.3)	0 (0.0)

CVD: Cardiovascular disease, HTN: Hypertension (High Blood Pressure), DM: Diabetes Mellitus, HLP: Hyperkeratosis Lenticularis Perstans (Flegel Disease), COPD: Chronic Obstructive Pulmonary Disease, MI: Myocardial Infarction, CVA: Cerebral Vascular Accident, RD: Renal Diseases

Table 2. Clinical presentations of study subjects

Variable	Category	Case (Total) N (%)
Cardiac & Respiratory Clinical signs	Shortness. Breath	22 (88)
	Chest. Pain	1 (4)
Neurology Clinical signs	Headache	4 (16)
	Vertigo	1 (4)
	Loss.Conscionusness	1 (4)
Digestive Clinical signs	Nausea	2 (8)
	Vomit	1 (4)
	Diarrhea	1 (4)
Systemic Clinical signs	Fever	8 (32)
	Cough	14 (56)
	Sore.Throut	1 (4)
	Shake	4 (16)
	Myalgia	4 (16)
	Anorexia	1 (4)

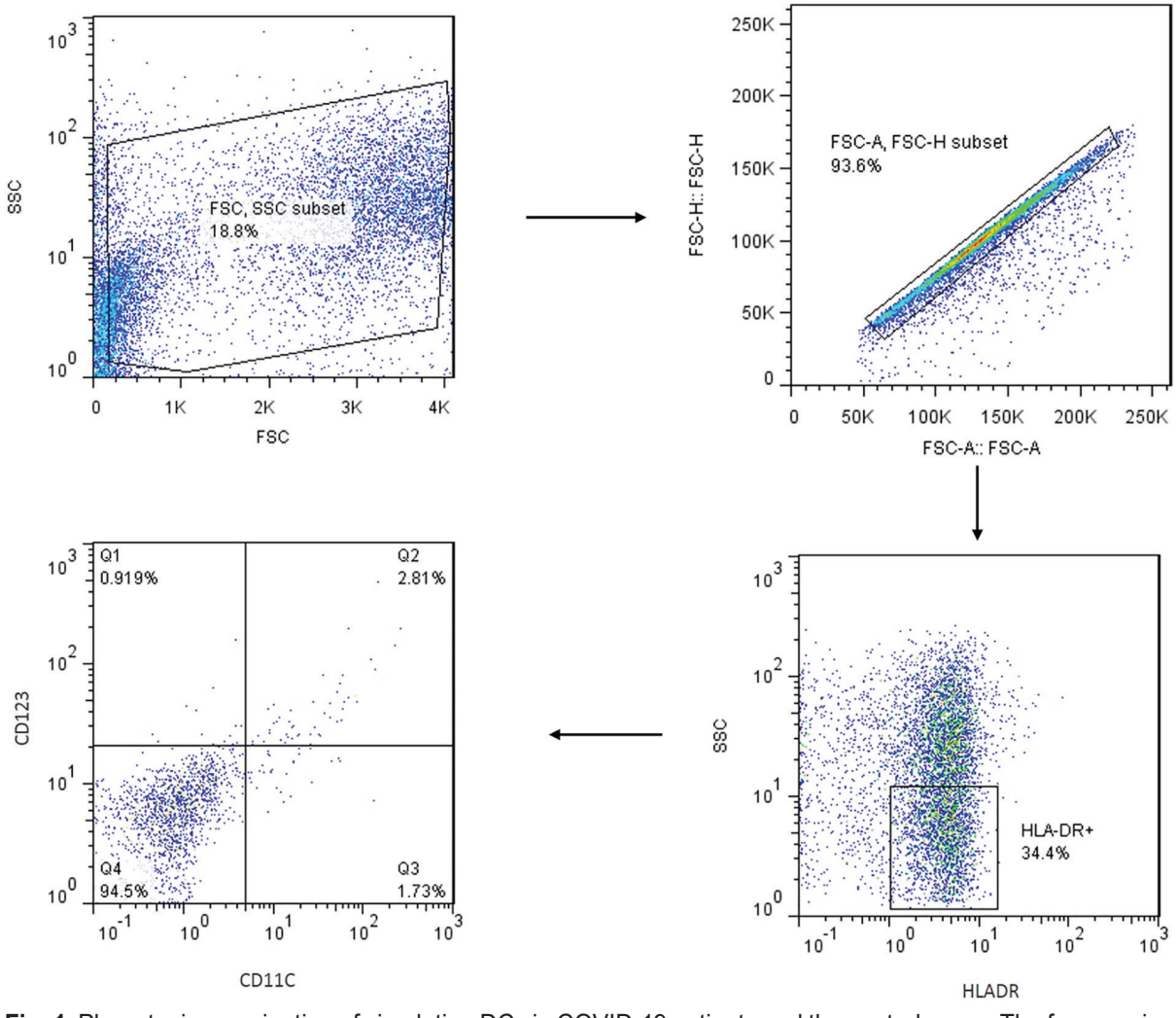

Fig. 1. Phenotypic examination of circulating DCs in COVID-19 patients and the control group. The frequencies and phenotypes of DCs in COVID-19 patients' PBMCs and healthy subjects were assessed using multi-color flow cytometry. The first gate was set on HLA-DR$^+$ cells and quantified as a percentage of gated cells by acquiring a minimum of 10,000 live events in each sample. The percentages of DCs subpopulations were as follows: CD123$^+$CD11c$^-$ pDCs, CD123$^-$CD11c$^+$ cDCs, and CD123$^+$CD11c$^+$ double-positive DCs

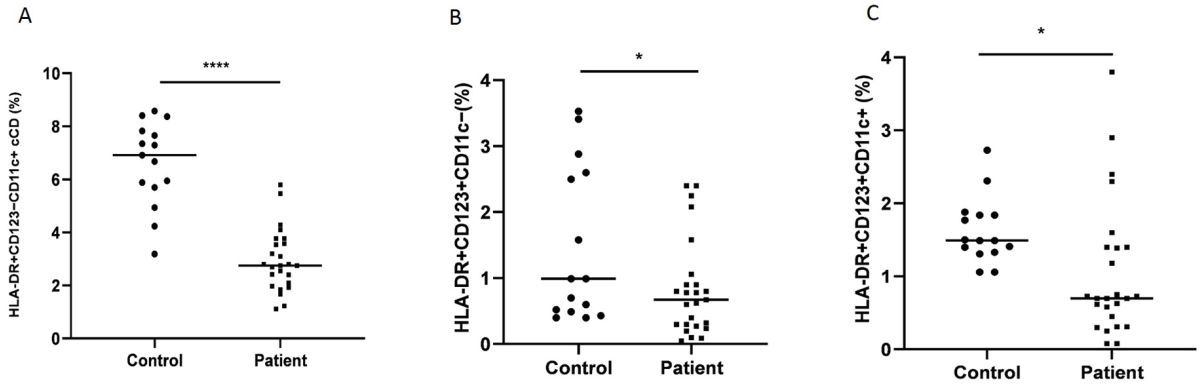

Fig. 2. Subpopulations of circulating DCs in study subjects. The analysis indicated that the means of cDCs, pDCs and double-positive DCs between control and case groups changed from 6.598% to 2.946%, 1.46% to 0.83% and 1.62% to 1.05% respectively *P≤0.05, **P≤0.01, ***P≤0.001, ****P≤0.0001

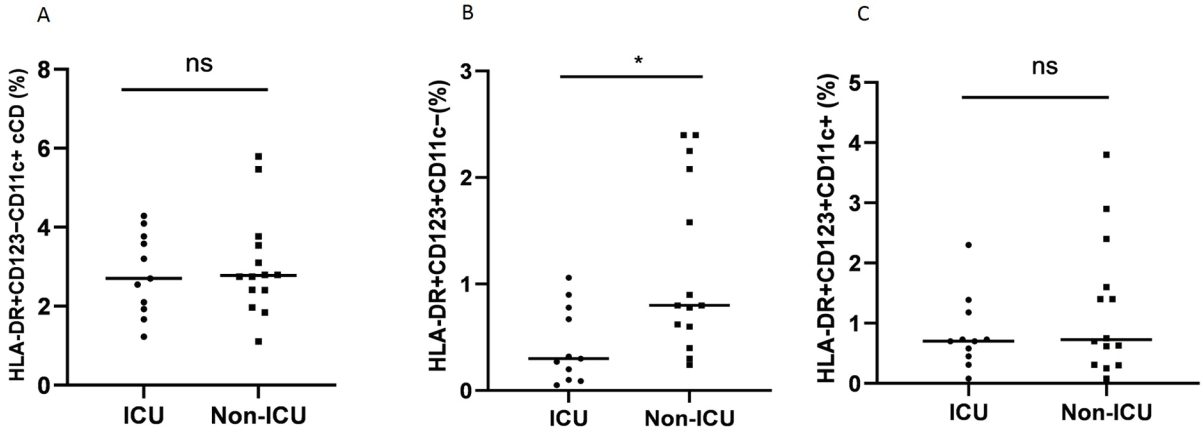

Fig. 3. Subpopulations of circulating DCs in patient according to ICU and non-ICU admission. Flow cytometric assessment of DC subtypes revealed a lower frequency of all subtypes of these cells (pDCs, cDCs, and dpDCs) in the peripheral blood of ICU COVID-19 patients compared to non-ICU admission. *P≤0.05, **P≤0.01, ***P≤0.001, ****P≤0.0001

The Distribution Profiles of DC Subsets Vary between Healthy Controls and COVID-19 Patients

The study examined the incidence of different types of DCs in the HLA-DR$^+$ population, comparing COVID-19 cases and healthy individuals (Fig. 2). Findings demonstrated a significant decline in the frequency of pDCs (0.83%) in the COVID-19 patients' peripheral blood compared to the healthy group (1.46%). Given that pDCs contribute to viral response by producing IFN-I, this decline may be linked to the severity of COVID-19. Additionally, the prevalence of CD123$^+$CD11c$^+$ double-positive DCs and CD123$^-$CD11c$^+$ cDCs was also significantly lower in COVID-19 patients (1.05% and 2.946%, respectively) compared to healthy controls (1.62%and 6.598%, respectively, P=0.03, and P<0.0001 respectively). Collectively, there were notable changes in the mean frequencies of cDCs (from 6.598% to 2.946%), pDCs (from 1.46% to 0.83%), and double positive DCs (from 1.62% to 1.05%) between the control and case groups.

The Distribution Profiles of DC Subsets Vary in COVID-19 Patients Based on Wether They Were Admitted in the ICU or Non-ICU

The distribution profiles of DC subclasses in COVID-19 patients were analyzed based on whether they were admitted to the ICU (Fig. 3). Flow cytometry assessments of peripheral blood pDC subtypes revealed a higher prevalence of these cells in non-ICU COVID-19 patients (1.15%) compared

to ICU COVID-19 patients (0.43%) with a significant difference (P<0.01). Additionally, the frequency of cDCs and double-positive DCs in the peripheral circulation was lower in ICU COVID-19 patients (2.82% for cDCs and 0.83% for dpDCs) compared to non-ICU patients (3.03% for cDCs and 1.22% for dpDCs). However, these differences were insignificant (P=0.66 and P=0.3, respectively).

The Distribution Profiles of DC Subclasses Vary between Healthy Controls and COVID-19 Patients Based on Para-clinical and Demographic Characteristics

The mean±SD and median paraclinical variables were measured for patients admitted to ICU and non-ICU wards, but a significant correlation could not be established between them. Table 3 provides a detailed breakdown of the results. Additionally, the variation of distribution profiles of all DC subsets was evaluated in both case and control groups based on demographic factors such as gender, age, blood type, and underlying conditions. Regarding age, all DC subtypes in COVID-19 patients were lower than healthy controls, regardless of whether they were above or below 50. Furthermore, all DC subtypes were reduced in both male and female groups, although the decrease in cDC and pDC groups was more pronounced in males than in females. However, no significant correlation was observed between these variables (see Table 4).

The Correlations between DC Subpoulations and Clinical Characteristics and Demographics of COVID-19 Patients

This study investigated the relationship between demographics, clinical characteristics and the ratio of DC subpopulations (cDC, pDC, and double positive DC) as detailed in Table 5. Our results suggest a significant association between the age of COVID-19 patients and cDC levels (P=0.049), as depicted in Fig. 4. Furthermore, we found a significant correlation between the MCHC variable and the level of double positive DC in COVID-19 patients (P=0.041), but not with other variables (Table 5).

Table 3. Para-clinical presentations of study subjects

Variable	mean±SD /median (IQR)	Grouped with ICU admission	Grouped with non-ICU admission	P value
WBC (cells/mm^3)	6360.00 (6050.00)	6200.00 (6100.00)	9350.00 (7475.00)	0.228
RBC (million/mm^3)	4.64±1.18	4.75±1.65	4.55±0.66	0.679
HB (g/dl)	12.98±1.99	12.66±2.22	13.23±1.84	0.489
HCT (%)	39.00±5.14	37.77±5.50	39.97±4.82	0.299
MCV (fl)	88.56±6.10	88.72±6.49	88.42±6.02	0.906
MCH (pg)	30.00 (3.50)	30.00 (3.00)	30.00 (4.25)	0.910
MCHC (g/dl)	33.52±2.21	33.90±2.50	33.21±2.00	0.449
PLT (cells/mm^3)	175338.16±88545.76	172454.54±84577.02	177603.85±94654.18	0.889
SGOT (IU/L)	62.00 (48.00)	53.00 (53.00)	73.00 (43.75)	0.208
SGPT (IU/L)	32.00 (44.50)	32.00 (48.00)	32.50 (50.00)	0.493
PT (sec)	13.00 (3.25)	12.50 (2.00)	14.20 (4.38)	0.102
INR (index)	1.10 (0.45)	1.00 (0.10)	1.20 (0.53)	0.420
PTT (sec)	38.00 (11.50)	37.00 (14.00)	39.00 (11.25)	0.869
Blood Sugar (mg/dl)	108.00 (40.00)	108.00 (37.00)	112.00 (54.75)	0.584
Urea	94.60±71.54	85.36±85.71	101.85±60.57	0.578
Creatinine (mg/dl)	1.20 (0.95)	1.20 (0.80)	1.20 (1.18)	0.742
LDH (IU/L)	1096.40±546.06	985.72±265.25	1183.35±691.27	0.380
CPK (IU/L)	180.00 (232.00)	145.00 (193.00)	191.00 (403.25)	0.565
ESR (mm/hr)	45.52±19.61	52.36±20.22	40.14±18.02	0.124
CRP (mg/ dl)	1.2±0.61	1.4±0.21	1±0.26	0.660

Table 4. Variation in distribution profiles of DC subsets in COVID-19 patients and healthy controls based on demographic characteristics

Variable	Group	cDC (%)		pDC (%)		dDC (%)	
		Case	Control	Case	Control	Case	Control
Age	≤ 50	1.81±0.59	6.30±1.78	0.31±.032	1.72±1.21	0.69±0.36	1.47±0.28
	> 50	3.16±1.12	6.85±1.49	0.93±0.75	1.24±1.17	1.12±0.99	1.76±0.54
	P value	0.422		0.120		0.810	
Sex	Male	2.50±0.96	6.59±1.35	0.92±0.90	1.42±1.14	0.74±0.48	1.64±0.53
	Female	3.35±1.22	6.60±2.19	0.75±0.56	1.54±1.36	1.33±1.15	1.58±0.25
	P value	0.356		0.654		0.218	
Blood type	A	2.92±0.93	6.43±1.69	0.79±0.89	1.17±1.11	0.91±0.74	1.80±0.52
	B	2.60±0.77	7.70±0.73	0.81±0.65	2.33±1.23	1.05±0.69	1.71±0.26
	AB	3.95±1.58	7.96±0.86	0.83±0.83	1.46±1.46	0.94±1.33	1.58±0.36
	O	2.77±1.56	4.96±0.73	0.94±0.79	1.29±1.37	1.32±1.38	1.17±0.19
	P value	0.132		0.522		0.595	
Underlying diseases	Yes	2.84±1.11	7.03±1.77	0.97±0.80	1.30±1.33	0.98±0.82	1.65±0.44
	No	3.20±1.34	6.30±1.50	0.47±0.33	1.57±1.12	1.21±1.21	1.61±0.48
	P value	0.251		0.240		0.645	

Table 5. Correlation of DC subpoulations with clinical characteristics and demographics of COVID-19 patients

Variable	cDC		pDC		DC.doblepositive	
	Pearson Correlation	P value	Pearson Correlation	P value	Pearson Correlation	P value
Age	0.382	0.049	0.268	0.190	0.131	0.52
WBC	0.209	0.317	-0.060	0.777	0.007	0.973
RBC	0.049	0.817	-0.098	0.643	-0.017	0.936
HB	-0.011	0.957	0.030	0.888	-0.081	0.701
HCT	0.102	0.627	0.123	0.558	0.086	0.683
MCV	-0.188	0.369	-0.072	0.732	-0.089	0.673
MCH	-0.351	0.085	-0.155	0.461	-0.371	0.068
MCHC	-0.319	0.120	-0.268	0.194	-0.412	0.041
PLT	0.473	0.017	0.038	0.859	0.207	0.320
SGOT	-0.190	0.362	-0.170	0.417	-0.128	0.543
SGPT	-0.128	0.543	-0.254	0.221	0.122	0.560
PT	-0.037	0.860	0.121	0.563	0.220	0.290
INR	-0.042	0.844	-0.094	0.656	0.169	0.418
PTT	0.141	0.502	0.071	0.736	0.295	0.152
Blood Sugar	-0.388	0.056	-0.154	0.463	-0.272	0.188
Urea	0.341	0.096	0.148	0.481	0.295	0.152
Creatinine	-0.085	0.685	-0.011	0.958	0.085	0.686
LDH	-0.153	0.466	-0.131	0.532	-0.130	0.537
CPK	-0.274	0.185	-0.199	0.340	-0.194	0.353
ESR	-0.020	0.924	-0.096	0.647	-0.116	0.581

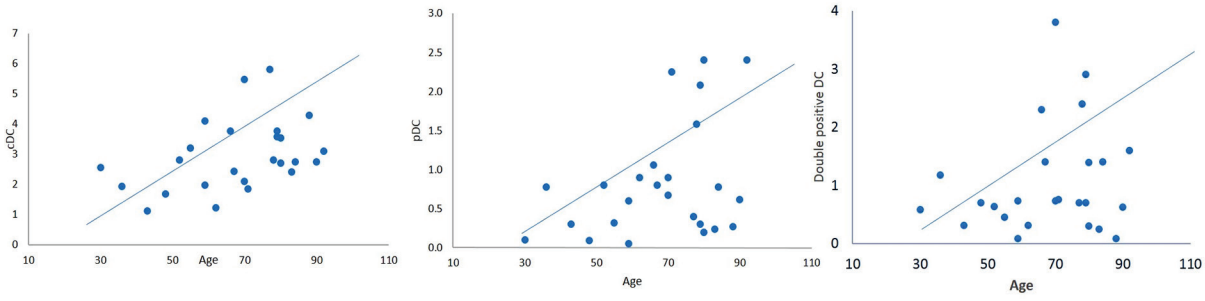

Fig. 4. DC subpopulations' correlations with aging. Based on the results, there was a significant correlation between the level of cDC and the age of COVID 19 patients

DISCUSSION

In our study, we investigated the potential changes in the occurrence of plasmacytoid DCs, conventional DCs, and double-positive DCs in the peripheral blood of COVID-19 patients. Our focus was on examining the expression levels of HLA-DR (MHC-II), CD123 (an IL-3 receptor), and CD11c on the surface of DCs. Our findings showed a decrease in all types of DCs in the COVID-19 group, with significant decline in cDCs and pDCs.

DCs are professional antigen-presenting cells that play an essential role in both specific and non-specific immunity against pathogen infections (14). These cells are present throughout the body, including the respiratory system (15). Conventional DCs help activate T cell ($CD8^+$ and $CD4^+$) responses by processing and presenting antigens to naïve T cells. On the other hand, pDCs are a significant source of IFN-1, showing potent anti-viral activity and a likely role in immune tolerance (2). A unique characteristic of pDCs is their high levels of expression of endosomal nucleic acid sensing Toll-like receptors (TLRs) TLR7 and TLR9, which bind to single-stranded RNA or DNA to detect viral pathogens and initiate an appropriate immune response (2).

Numerous studies have confirmed the significant role that DCs play in the pathogenesis and severity of SARS-CoV-2 infection (14, 16). As mentioned earlier, the expression of ACE-2 by lung' DCs serves as a critical receptor for SARS-CoV-2 allowing the virus to enter host cells through interaction with the spike protein, leading to in infection (15). The likelihood of infection is also increased through interaction with the dipeptidyl peptidase (DPP) 4 receptor or microcellular proliferation (17, 18). Despite studies investigating the mechanisms ibehind the dysfunction of dendritic cells infected with SARS-CoV, the details of the interaction between DCs and SARS-CoV-2 remained unclear. In one study, DCs infected with SARS-CoV were fluorescently stained revealing early infection and the presence of negative strands of viral RNA in DCs, indicating the virus's ability to replicate within these cells. However, the replication of the virus within DCs was found to be imperfect and incomplete (19-21).

It has been proposed that, SARS-CoV-2 displays a cytopathic effect on DCs and is considered one of the main mechanisms driving the decreases and impairment in the number and function of DCs. It was demonstrated that IFN γ – α and IFN-β production was significantly disrupted by $CD11c^+$ conventional DCs in patients with acute coronavirus infection (19). Moreover, expression of costimulatory molecules B7-1 and B7-2 were diminished in SARS-CoV infected cDCs, therefore, $TCD4^+$ and $TCD8^+$ cells expansion and function against SARS-CoV-2 were also weakened. On the other hand, the antigen presentation capacity of DCs was impaired due to the diminished expression of HLA-DR in virus infected DCs (22).

Some risk factors, such as age, sex,

comorbidities, and the status of the immune cells and response could determine the disease outcome after infection (23). The immune response dysregulation has been reported in COVID19 patients who progress to severe disease, as identified by inflammatory responses and subsequent tissue damage in organs especially the lungs. In milder forms of the disease, the immune system can manage the infection without causing known organ damage. The observed impairment of DC function results in a diminished ability to stimulate T cells and a reduced capacity to secrete antiviral cytokines (24). In patients with severe COVID-19, several reports have concluded that Type I and III IFN responses are inhibited during the early phase of the infection (25). pDCs are known as one of the primary first-line defenders that recognize and respond to the viruses , with the ability to produce IFN-I and III (26). In patients with COVID-19, there is a decrease in the number and function of DCs and more importantly an IFN-I deficiency resulting in SARS-CoV-2 escape. This elicits immune cells to produce ineffective proinflammatory cytokines, thus initiating a cytokine storm. Severe forms of COVID-19 disease have been reported in individuals with a deficiency in Type I interferon signaling. It has been shown that preexisting auto-antibodies against Type I IFNs in patients with Autoimmune Polyglandular Syndrome Type 1 (APS-1) make them more susceptible to severe forms of COVID-19 throughout their lifespan. These data reinforce the critical role of DCs and IFN-I signaling as the first line of protection against COVID-19 (27).

On the other hand, rapid reduction in DC frequency and functionality may cause delayed T-cell immune responses among COVID-19 patients (15). An experiment by Zhou and colleagues provides evidence that acute COVID-19 infection can impair the functionality of host T and dendritic cells. This impairment may contribute to the severity of the virus infection and its pathogenesis (19). Another study revealed that phenotypic impairment and depletion of circulating DCs during the disease's acute phase could lead to vulnerability to more severe symptoms, prolonged inflammatory response, and secondary infections (28). Similarly, Parackova et al. demonstrated that hyperinflammation in COVID-19 is associated with augmented neutrophils and impaired functionality of monocytes and dendritic cells (29). Consequently, these functional defects affect the infection outcomes, clinicopathological features, and future re-exposure. Furthermore, these DC alterations affect immune memory, as well as vaccine efficacy.

Several studies in murine norovirus (MNV) infection have shown that depletion of conventional DCs results in increased viral loads in intestinal tissues, a weaker generation of antibody responses, and a failure of MNV to efficiently infect lymphoid tissues (30). These changes in COVID-19 patients may reflect a regulatory mechanism to reduce overactivation of the immune response. The defined alterations combined with severe lymphopenia could make patients more susceptible to secondary infections, which have been shown to be more widespread in COVID-19 patients. This needs to be taken into consideration in the clinical management of COVID-19 (21).

Saichi and colleagues, using single-cell RNA sequencing, found that APCs in the blood of individuals with severe or moderate COVID-19 showed viral inhibition of antigen presentation (31). They also observed a decrease in the DHX36 and TLR9 innate sensors in CLEC9a^{+} DCs and pDCs, respectively, an incraese in pro-apoptotic pathways in pDCs, a reduction in MHC class II trans activator activity and MHC class II-related genes in cDC1c^{+} DCs and downregulation of anti-viral interferon-stimulated genes in monocyte subtypes (31). Our data supports these findings. Notably, IFN-neutralizing Abs in COVID-19 patients have been linked to downregulation of the IFN response and increased disease severity,

especially in male patients (6). Similarly, another study found that fatal COVID-19 cases were associated with decreased expression of CD123, CD11c, or HLA-DR on circulating DCs and a lower frequency of pDC compared to other subtypes (32). Our findings are consistent with previous research on COVID-19 patients.

The current cross-sectional descriptive study, showed a significant decline in the frequency of pDCs in the peripheral blood of COVID-19 patients compared to the healthy group. This decrease was more pronounced in patients admitted to the ICU and the percentage of DCs was associated with the severity of COVID-19. If a longitudinal study were conducted, we could determine the relationship between changes in the frequency of DCs and the patient's condition, including the recovery process. Therfore, the following suggestions for future studies are proposed: conducting a study with a larger sample size and including different age groups, investigating patients with various underlying diseases, conducting longitudinal studies with repeated measurements at different stages (such as the onset,peak,and the recovery stages of the disease) to provide information about the dynamics of the measured cells, comparing results with other viral diseases, and examining the effect of different treatments on the distribution of the studied cells.

CONCLUSION

Our study reveals a significant decrease in peripheral blood DC frequencies among COVID-19 patients, which may be due to the virus infecting and damaging these cells. This results in the immediate suppression of the host's innate immune response against the infection, potentially worsening the severity of the disease. Additionally, we observed a significant decrease in DCs among ICU patients compared to non-ICU patients, indicating a correlation between DC frequency and disease severity. Therefore, SARS-CoV-2 can evade immune response attacks by reducing the number of DCs and suppressing their function leading to a weakened development of both innate and adaptive immunity. As a result, targeting the number and function of DCs for therapeutic purposes, could enhance immune responses in COVID-19 patients.

ACKNOWLEDGMENT

The Students Research Committee at Ardabil University of Medical Sciences has supported this study. The authors are grateful to the patients and healthy individuals who participated in the study. Additionally, the authors would like to acknowledge the valuable assistance provided by the staff at Imam Khomeini Hospital in Ardabil, Iran.

FUNDING

This study was supported by Ardabil University of Medical Sciences (finance code1003975).

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study conducted at Ardabil University of Medical Sciences in Iran received the necessary approval from the Ethics Committee (IR.ARUMS.REC.1399.038) and all patients provided their informed consent as required.

AUTHORS' CONTRIBUTION

Elham Safarzadeh and Afshin Fathi conceived and planned the study; Vahid Asghariazar, Majid Eterafi, and Somaieh Matin carried out the experiment and collected relevant literature, Rozita Abolhasani, Monireh

Falsafi, and Elham Safarzadeh, prepared the manuscript, Nasrin Fouladi analyzed the statistical data and verified the accuracy of the tests.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Cabeza-Cabrerizo M, Cardoso A, Minutti CM, Pereira da Costa M, Reis e Sousa C. Dendritic cells revisited. Annual review of immunology. 2021;39(1):131-66.
2. Falck-Jones S, Österberg B, Smed-Sörensen A. Respiratory and systemic monocytes, dendritic cells, and myeloid-derived suppressor cells in COVID-19: Implications for disease severity. Journal of Internal Medicine. 2023;293(2):130-43.
3. Belderbos RA, Aerts JG, Vroman H. Enhancing dendritic cell therapy in solid tumors with immunomodulating conventional treatment. Molecular Therapy-Oncolytics. 2019;13:67-81.
4. Böttcher JP, e Sousa CR. The role of type 1 conventional dendritic cells in cancer immunity. Trends in cancer. 2018;4(11):784-92.
5. Asselin-Paturel C, Trinchieri G. Production of type I interferons: plasmacytoid dendritic cells and beyond. The Journal of experimental medicine. 2005;202(4):461-5.
6. Frasca F, Scordio M, Santinelli L, Gabriele L, Gandini O, Criniti A, et al. Anti-IFN-α/-ω neutralizing antibodies from COVID-19 patients correlate with downregulation of IFN response and laboratory biomarkers of disease severity. European Journal of Immunology. 2022;52(7):1120-8.
7. Neyt K, Lambrecht BN. The role of lung dendritic cell subsets in immunity to respiratory viruses. Immunological reviews. 2013;255(1):57-67.
8. Dress RJ, Wong AY, Ginhoux F. Homeostatic control of dendritic cell numbers and differentiation. Immunology and cell biology. 2018;96(5):463-76.
9. Ciotti M, Ciccozzi M, Terrinoni A, Jiang W-C, Wang C-B, Bernardini S. The COVID-19 pandemic. Critical reviews in clinical laboratory sciences. 2020;57(6):365-88.
10. Eterafi M, Makaremi S, Shaker H, Fouladi N, Shahgoli VK, Jeddi F, et al. Demographic, Clinical, and Paraclinical Characteristics of the Fourth Surge of the COVID-19 Pandemic. Journal of Inflammatory Diseases. 2024;26(3).
11. Chowdhury MA, Hossain N, Kashem MA, Shahid MA, Alam A. Immune response in COVID-19: A review. Journal of infection and public health. 2020;13(11):1619-29.
12. Hue S, Beldi-Ferchiou A, Bendib I, Surenaud M, Fourati S, Frapard T, et al. Uncontrolled innate and impaired adaptive immune responses in patients with COVID-19 acute respiratory distress syndrome. American journal of respiratory and critical care medicine. 2020;202(11):1509-19.
13. Kumar R, Rathi H, Haq A, Wimalawansa SJ, Sharma A. Putative roles of vitamin D in modulating immune response and immunopathology associated with COVID-19. Virus research. 2021;292:198235.
14. Sanchez-Cerrillo I, Landete P, Aldave B, Sánchez-Alonso S, Azofra AS, Marcos-Jiménez A, et al. Differential redistribution of activated monocyte and dendritic cell subsets to the lung associates with severity of COVID-19. MedRxiv. 2020:2020.05. 13.20100925.
15. Galati D, Zanotta S, Capitelli L, Bocchino M. A bird's eye view on the role of dendritic cells in SARS-CoV-2 infection: Perspectives for immune-based vaccines. Allergy. 2022;77(1):100-10.
16. Campana P, Parisi V, Leosco D, Bencivenga D, Della Ragione F, Borriello A. Dendritic cells and SARS-CoV-2 infection: still an unclarified connection. Cells. 2020;9(9):2046.
17. Lan J, Ge J, Yu J, Shan S, Zhou H, Fan S, et al. Structure of the SARS-CoV-2 spike receptor-binding domain bound to the ACE2 receptor. nature. 2020;581(7807):215-20.
18. Gao C, Zeng J, Jia N, Stavenhagen K, Matsumoto Y, Zhang H, et al. SARS-CoV-2 spike protein interacts with multiple innate immune receptors. BioRxiv. 2020.
19. Zhou R, To KK-W, Wong Y-C, Liu L, Zhou B, Li X, et al. Acute SARS-CoV-2 infection impairs dendritic cell and T cell responses. Immunity. 2020;53(4):864-77. e5.
20. Silvin A, Chapuis N, Dunsmore G, Goubet A-G, Dubuisson A, Derosa L, et al. Elevated calprotectin and abnormal myeloid cell subsets discriminate severe from mild COVID-19. Cell. 2020;182(6):1401-18. e18.
21. Wang X, Guan F, Miller H, Byazrova MG, Candotti F, Benlagha K, et al. The role of dendritic cells in COVID-19 infection. Emerging Microbes & Infections. 2023;12(1):2195019.
22. Shi W, Liu X, Cao Q, Ma P, Le W, Xie L, et al. High-dimensional single-cell analysis reveals the immune characteristics of COVID-19. American Journal of Physiology-Lung Cellular and Molecular Physiology. 2021;320(1):L84-L98.
23. Zhang J-j, Dong X, Liu G-h, Gao Y-d. Risk

and protective factors for COVID-19 morbidity, severity, and mortality. Clinical reviews in allergy & immunology. 2023;64(1):90-107.
24. Winheim E, Eser T, Deák F, Ahmed MI, Baranov O, Rinke L, et al. Distinct and dynamic activation profiles of circulating dendritic cells and monocytes in mild COVID-19 and after yellow fever vaccination. European Journal of Immunology. 2023;53(3):2250090.
25. Park A, Iwasaki A. Type I and type III interferons–induction, signaling, evasion, and application to combat COVID-19. Cell host & microbe. 2020;27(6):870-8.
26. Reizis B. Plasmacytoid dendritic cells: development, regulation, and function. Immunity. 2019;50(1):37-50.
27. Rajamanickam A, Kumar NP, Pandiaraj AN, Selvaraj N, Munisankar S, Renji RM, et al. Restoration of dendritic cell homeostasis and Type I/Type III interferon levels in convalescent COVID-19 individuals. BMC immunology. 2022;23(1):51.
28. Winheim E, Rinke L, Lutz K, Reischer A, Leutbecher A, Wolfram L, et al. Impaired function and delayed regeneration of dendritic cells in COVID-19. PLoS pathogens. 2021;17(10):e1009742.
29. Parackova Z, Zentsova I, Bloomfield M, Vrabcova P, Smetanova J, Klocperk A, et al. Disharmonic inflammatory signatures in COVID-19: augmented neutrophils' but impaired monocytes' and dendritic cells' responsiveness. Cells. 2020;9(10):2206.
30. Elftman MD, Gonzalez-Hernandez MB, Kamada N, Perkins C, Henderson KS, Nunez G, et al. Multiple effects of dendritic cell depletion on murine norovirus infection. Journal of General Virology. 2013;94(8):1761-8.
31. Saichi M, Ladjemi MZ, Korniotis S, Rousseau C, Ait Hamou Z, Massenet-Regad L, et al. Single-cell RNA sequencing of blood antigen-presenting cells in severe COVID-19 reveals multi-process defects in antiviral immunity. Nature Cell Biology. 2021;23(5):538-51.
32. Hasan A, Al-Ozairi E, Hassan NY, Ali S, Ahmad R, Al-Shatti N, et al. Fatal COVID-19 is Associated with Reduced HLA-DR, CD123 or CD11c Expression on Circulating Dendritic Cells. Journal of Inflammation Research. 2022:5665-75.