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# Evaluation of Myeloid-derived Suppressor Cells in the Blood of Iranian COVID-19 Patients

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## ABSTRACT

The cytokine storm and lymphopenia are reported in coronavirus disease 2019 (COVID-19). Myeloid-derived suppressive cells (MDSCs) exist in two different forms, granulocyte (G-MDSCs) and monocytic (M-MDSCs), that both suppress T-cell function. In COVID-19, the role of chemokines such as interleukin (IL)-8 in recruiting MDSCs is unclear. A recent report has correlated IL-8 and MDSCs with poor clinical outcomes in melanoma patients. In the current study, we evaluated the frequency of MDSCs and their correlation with serum IL-8 levels in severe COVID-19 patients from Iran.

Thirty-seven severe patients (8 on ventilation, 29 without ventilation), thirteen moderate COVID-19 patients, and eight healthy subjects participated in this study between 10th April 2020 and 9th March 2021. Clinical and biochemical features, serum, and whole blood were obtained. CD14, CD15, CD11b, and HLA-DR expression on MDSCs was measured by flow cytometry.

COVID-19 patients compared to healthy subjects had a greater frequency of M-MDSCs (12.7±13.3% vs 0.19±0.20%,), G-MDSCs (15.8±12.6% vs 0.35±0.40%,) and total-MDSCs (27.5±17.3% vs 0.55±0.41%,). M-MDSC (16.8±15.8% vs 5.4±4.8%,) and total-MDSC (33.3±18.5% vs 17.3±13.3%) frequency was higher in non- ventilated compared to moderate COVID-19 subjects. Serum IL-8 levels were higher in patients with COVID-19 than in normal healthy subjects (6.4±7.8 vs. 0.10±00 pg/mL). Ventilated patients (15.7±6.7 pg/mL),

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non-ventilated patients (5.7±2.7 pg/mL) and moderate patients (2.8±3.0 pg/mL) had significantly different levels of IL-8. A negative correlation was found between the frequency of G-MDSCs and the international normalized ratio (INR) test (r=-0.39), and between the frequency of total-MDSCs and oxygen saturation (%) (r=-0.39).

COVID-19 patients with severe non-ventilated disease had the highest levels of M-MDSCs. In addition to systemic MDSCs, lung, serum IL-8, and other inflammatory biomarkers should be measured.

**Keywords:** Blood; COVID-19; Interleukin-8; Myeloid-derived suppressor cells; Serum

## INTRODUCTION

Infections due to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) rapidly reached pandemic levels after its initial detection in December 2019 in Wuhan, China.[1] Clinically, infection with SARS-CoV-2, or coronavirus disease 2019 (COVID-19), results in a range of severity from asymptomatic infection to a very severe acute respiratory distress syndrome (ARDS)-like disease requiring ventilation with, some patients having mild respiratory symptoms with a dry cough and fever. Viral transmission occurs through human-to-human contact.[2,3] with a mortality rate up to 50-65% in ICU patients with severe COVID-19.[4] Respiratory pneumonia is a major complication of severe SARS-CoV-2 infection. However, the pathophysiology of severe COVID-19 is variable.[5]

Biomarkers that accurately and rapidly predict COVID-19 prognosis are required. Investigations of the immune profiles of SARS-COV1 and Middle East respiratory syndrome coronavirus (MERS-COV) indicate the critical role of lymphopenia and enhanced immune responses in disease severity.[6-8] Effective treatment of patients with severe disease requires deep molecular and immune phenotyping to understand the complexities of severe COVID-19. Myeloid-derived suppressive cells (MDSCs) originate from the myeloid-cell lineage and are a diverse group of relatively immature myeloid cells (IMCs).[9] Interleukin (IL)-6 and IL-8 concentrations are essential in melanoma; IL-6 potentially expands peripheral MDSCs, and IL-8 recruits these cells to the tumor microenvironment.[10]

The mechanisms by which MDSCs induce immune suppression have been reported in several studies.[9] MDSCs may be sub-grouped according to whether they are granulocytic (G-MDSCs) or monocytic (M-MDSCs). IMCs are generated in the bone marrow of normal healthy subjects before subsequently differentiating into mature granulocytes, macrophages, or dendritic cells (DCs).[7] The expansion of MDSCs is reported in several diseases, including sepsis, trauma, bone marrow transplantation, and some autoimmune diseases, as well as in cancer due to high levels of concurrent inflammation. They act to suppress immunomodulatory T-cell functions leading to heightened immune responses.[9,11,12] G-MDSCs are associated with generating highly reactive oxygen species (ROS) and low nitric oxide (NO) levels, while high NO and low ROS levels are seen in the M-MDSC subset. Both G-MDSCs and M-MDSCs produce arginase.[13] Studies in both animals and humans demonstrate that MDSCs regulate cytokine production[14] and may enhance cancer metastasis.[13,15]

However, a beneficial role for MDSCs has also been described during acute inflammatory responses to dengue virus infection, leading to reduced inflammation and attenuation of immune-mediated disease pathology.[16] Interestingly, an expansion in the systemic population of both G-MDSC and M-MDSC subtypes is observed in subjects with severe COVID-19.[7,17-19] However, these observations must be validated in cohorts with distinct ethnic and/or genetic backgrounds. We hypothesized that MDSC populations and their ability to evoke immune suppression vary according to COVID-19 severity in Iranian subjects. Therefore, we investigated the numbers of peripheral blood M-MDSCs and G-MDSCs in Iranian issues with moderate and severe COVID-19 and examined their correlation with markers of systemic inflammation such as IL-8.

## MATERIALS AND METHODS

### Patients

Fifty confirmed COVID-19 patients, including 8 severe patients on ventilation, 29 severe non-ventilated, and 13 moderate patients, participated in the study upon

admission to the Masih Daneshvari Hospital of Shahid Beheshti Medical University (Tehran-Iran) between 10th April 2020- 9th March 2021. All patients were diagnosed as stated by World Health Organization interim guidance.[20] The patients with severe COVID-19 were confirmed by at least one of the following criteria: respiratory rate≥30/min; blood oxygen saturation ≤93%; ratio of partial pressure of oxygen in arterial blood to the inspired oxygen fraction (PaO2FiO2) <300; lung infiltrates present on>50% of the lung field.[21] Eight healthy age-matched controls were also recruited. Masih Daneshvari Hospital's ethical committee approved this study (IR.SBMU.NRITLD.REC.1399.122).

**Data Collection**

The clinical records of patients were collected from electronic medical records from Masih Daneshvari Hospital. The information Frecorded included demographic data, medical history, underlying comorbidities, symptoms, signs, laboratory findings, chest computed tomographic (CT) scans, and treatment measures including antiviral therapy, corticosteroid therapy, respiratory support, and kidney replacement therapy.

**Laboratory Examination of Blood Samples**

Whole blood samples containing anti-coagulant EDTA (3 mL) and citrate (3 mL) or no anticoagulant (3 mL) were obtained from all participants upon admission. Tubes containing blood without anticoagulants were centrifuged, and the serum was separated and stored at -80°C for IL-8 measurement. Serum tests including kidney and liver function tests: creatinine kinase-muscle/brain activity (CK-MB), lactate dehydrogenase (LDH), C-reactive protein (CRP), ferritin, creatine phosphokinase (CPK), and the international normalized ratio (INR) were also performed. The erythrocyte sedimentation ratio was determined in citrate-treated whole blood samples.

**Cell Staining and Flow Cytometry**

Three mL of whole blood in EDTA were obtained from all participants and MDSCs were analyzed by flow cytometry (FACS Calibour, BD, USA) as described earlier.[22, 23] Briefly, blood cells were stained with anti-CD11b APC (BD Biosciences, CA, USA), anti-HLA-DR PE (eBioscience, San Diego, CA, USA), anti-CD14 PerCP-cy5.5 (eBioscience) and anti-CD15 FITC (BD Biosciences) for 30 min in the dark as described before.[22,24] Cells were washed and suspended in FACS buffer before being analyzed by FACS with 20,000 events being recorded. The gating strategy was CD11b+/HLA-DR-/dim, and within this population, CD14+/CD15- cells and CD14-/CD15+ were identified as described previously.[22,24] Flow cytometry data were subsequently analyzed by FlowJO-V10 software (USA) and reported as the frequency (percentage) of the respective subset of leukocytes.

**Measurement of IL-8**

Serum concentrations of IL-8 were measured by enzyme-linked immunoassay (ELISA) (BD Biosciences, CA, USA) as described in the Manufacturer's datasheet.

**Statistical Analysis**

Data analysis was performed using the SPSS version 16.0 and Graph Pad Prism version 6. Data were assessed for normal distribution. Differences between multiple groups were compared using a one-way analysis of variance (ANOVA), while the non-parametric Mann-Whitney U test was used for non-normally distributed variables. If the ANOVA provided $p<0.05$, then differences between the two selected groups were analyzed using Student's *t*-test. A Pearson correlation analysis was applied to measure the linear correlation between two data sets, and a chi-square test was used for sex comparisons between different groups. Results reported as the mean±standard deviation (SD) and $p<0.05$ was considered statistically significant.

## RESULTS

**Demographic Information of Patients with COVID-19 and Healthy Control Subjects**

The demographic information of the participants is shown in Table 1. The serum levels of ESR, CRP, LDH, Troponin, and MB activity between ICU (ventilated and non-ventilated) and non-ICU moderate COVID-19 patients showed no significant differences. However, CPK was higher in ICU patients (ventilated and non-ventilated) than in non-ICU moderate patients ($p=0.01$) (Table 1).

**MDSC Analysis**

The frequency of M-MDSCs (HLA-$DR^{/dim}CD11b^{+}CD14^{+}CD15^{-}$ cells) and G-MDSCs (HLA-$DR^{-/dim}CD11b^{+}CD14^{-}CD15^{+}$ cells) in the blood

were evaluated as described previously.[24] The gating strategy way and the frequency of G-MDSC and M-MDSC cells in representative healthy control subjects and COVID-19 patients of differing severity are shown in Figure 1. There was a significantly greater frequency of M-DSCs (Figure 2A, Table 2), G-MDSCs (Figure 2B, Table 2), and total-MDSC (Figure 2C, Table 2) in all COVID-19 patients as a group compared to healthy subjects ($p \leq 0.0001$, $p \leq 0.0001$, $p \leq 0.0001$ respectively).

Next, we analyzed the M-MDSC, G-MDSC, and total-MDSC cells in moderate and severe COVID-19 patients. ANOVA showed significant differences in the frequency of M-MDSCs between moderate, non-ventilated, and ventilated patients ($p$=0.03) (Figure 2D, Table 3). In addition, there was a significant increase in M-MDSC frequency in COVID-19 patients who were not ventilated compared to moderate patients ($p$=0.004) (Figure 2D, Table 3). In contrast, no significant differences were found between ventilated and non-ventilated patients in the frequency of M-MDSC (Figure 2D, Table 3). Furthermore, moderate and severe COVID-19 patients have a significantly higher frequency of M-MDSC compared with HC (Figure 2D, Table 3).

**Table 1. The demographic data and biochemical characters of participants**

| | ***Severe (S)*** | | **M (Non-ICU)** | **HC** | ***P*** | |
|---|---|---|---|---|---|---|
| | **V N=8** | **NV N=29** | **N=13** | **N=8** | **ICUvs. N-ICU** | **V vs. NVS vs. M** |
| **Age, years** | 62.1±9.5 | 58.3±13.2 | 50.0±16.8 | 40.1±10 | **0.04** | 0.10 |
| **Female, N (%)** | 5(62.5) | 13(44.8) | 7(53.8) | 1 (12.5) | 0.74 | 0.64 |
| **Male, N (%)** | 3(37.5) | 16(55.2) | 6(46.2) | 7 (87.5) | | |
| **ESR (mm/hr)** | 52.0±31.9 N=7 | 35.3±29.1 N=21 | 44.0±37.3 N=12 | - | 0.69 | 0.46 |
| **CRP (mg/L)** | 26.7±12.4 N=4 | 40.6±25.6 N=22 | 40.0±19.11 N=4 | - | 0.91 | 0.56 |
| **LDH (U/L)** | 677.1±459.1 N=7 | 577.1±253.6 N=26 | 492.9±124.4 N=11 | - | 0.27 | 0.37 |
| **Troponin (pg/mL)** | 0.02±0.0 N=4 | 0.11±0.43 N=22 | 0.02±0.0 N=3 | - | 0.74 | 0.87 |
| **CPK (U/L)** | 246.1±301.6 N=7 | 215.4±295.9 N=26 | 85.1±63.23 N=10 | - | **0.01** | 0.35 |
| **D-Dimer (ng/mL)** | 556.5±456.0 N=2 | 1167.8±1351.1 N=22 | 3945.0±4320.4 N=2 | - | 0.52 | 0.06 |
| **CK-MB Activity (U/L)** | 45.6±36.6 N=3 | 44.7±48.1 N=19 | 40.0±16.9 N=2 | - | 0.88 | 0.98 |
| **PaO2FiO2 %** | 87.3±7.8 N=3 | 65.5±23.2 N=22 | 75.0±24.4 N=4 | - | 0.58 | 0.27 |
| **INR** | 1.1±0.05 N=4 | 1.1±0.12 N=23 | 1.1±0.13 N=4 | - | 0.43 | 0.60 |

*****Values were presented as Mean ± SD. p<0.05 was considered significant

N numbers are for the whole group unless otherwise indicated. CK-MB: creatinine kinase-muscle/brain; CPK: creatine phosphokinase CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; INR: international normalized ratio; LDH: lactate dehydrogenase; PaO2/FiO2 %: percentage of arterial oxygen partial pressure (PaO2) to fractional inspired oxygen (FiO2; HC: Healthy control; M: Moderate; S: Severe; ICU: Intensive care unit; NV: Non-ventilated; V: Ventilated

No significant differences in G-MDSC cell frequency were found between moderate and severe COVID-19 patients, although all COVID-19 groups had a higher frequency than observed HC (Figure 2E, Table 3). Significant differences in the frequency of total-MDSCs between moderate, non-ventilate, and ventilated patients were observed ($p$=0.018) (Figure 2F). In addition, there was a significant increase in total MDSC frequency in severe COVID-19 patients who were not ventilated compared to moderate patients ($p$=0.009) (Figure 2E, Table 3). No significant differences were found between ventilated and non-ventilated patients in the frequency of total MDSC (Figure 2E). Furthermore, moderate and severe COVID-19 patients have a significantly higher frequency of total MDSC compared with HC (Figure 2E, Table 3).

**IL-8 Levels**

IL-8 levels in the serum of COVID-19 patients (moderate and severe) were significantly higher than in healthy control subjects ($p$=0.03) (Figure 3A, Table 2). In addition, IL-8 levels in serum were significantly higher in severe COVID-19 patients on ventilation compared to those who were not ventilated ($p$=0.04) and in moderate patients ($p$=0.008) (Figure 3B, Table 3). Systemic IL-8 levels were higher in non-ventilated patients than in patients with moderate COVID-19 ($p$=0.01) (Figure 3B, Table 3).

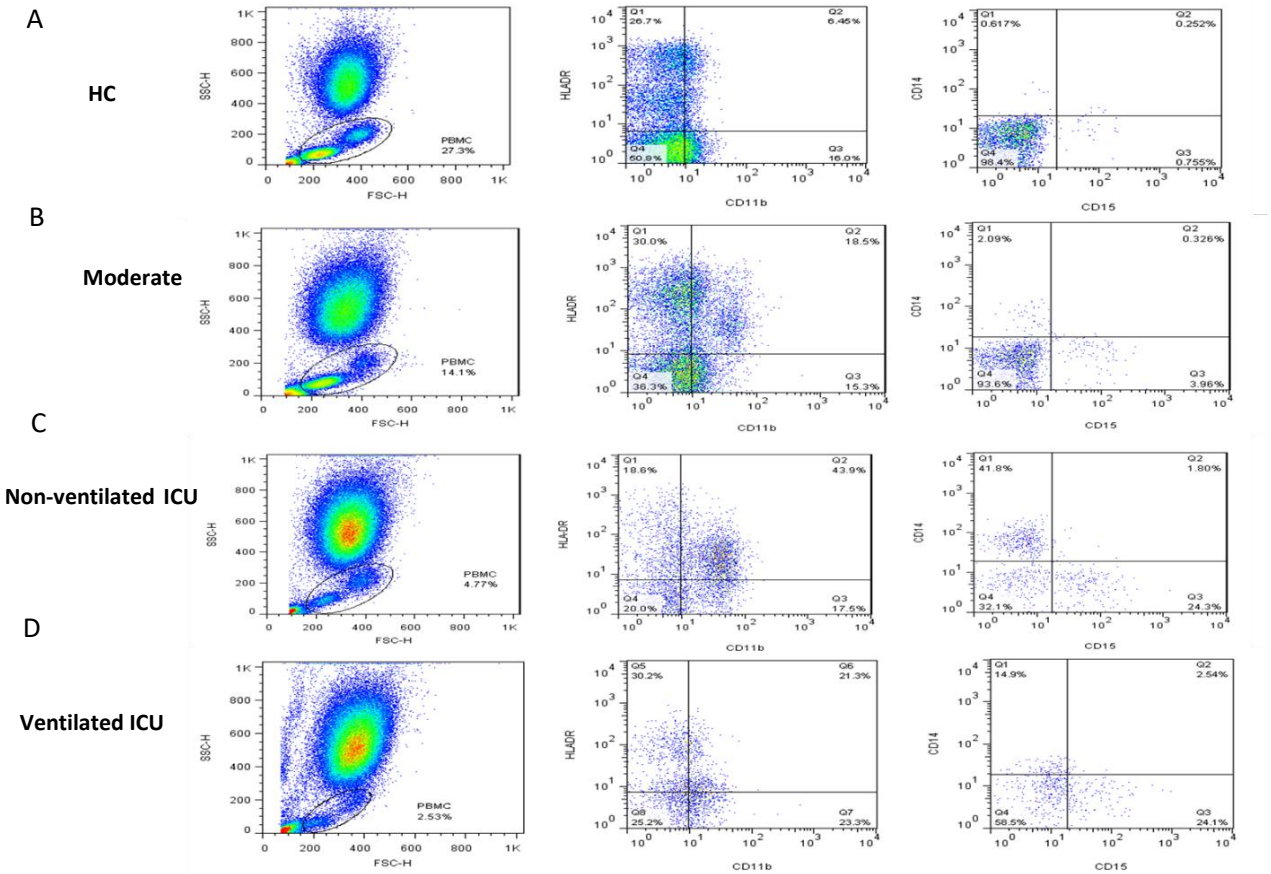


**Figure 1. Gating strategy for monocytic (M)-myeloid-derived suppressive cells (MDSC) and granulocytic (G)-MDSC cells. Representative dot plots of flow cytometry data M-MDSCs (HLA-DR$^{-/dim}$CD11b$^{+}$CD14$^{+}$CD15$^{-}$ cells) and G-MDSCs (HLA-DR$^{-/dim}$CD11b$^{+}$CD14$^{-}$CD15$^{+}$ cells) in the blood of a healthy control (HC) subject (A) and moderate (B) non-ventilated intensive care unit (ICU) (C) and ventilated ICU (D) patient's frequency of cells are indicated in the text boxes in the top left (M-MDSC) and bottom right (G-MDSC) panels.**

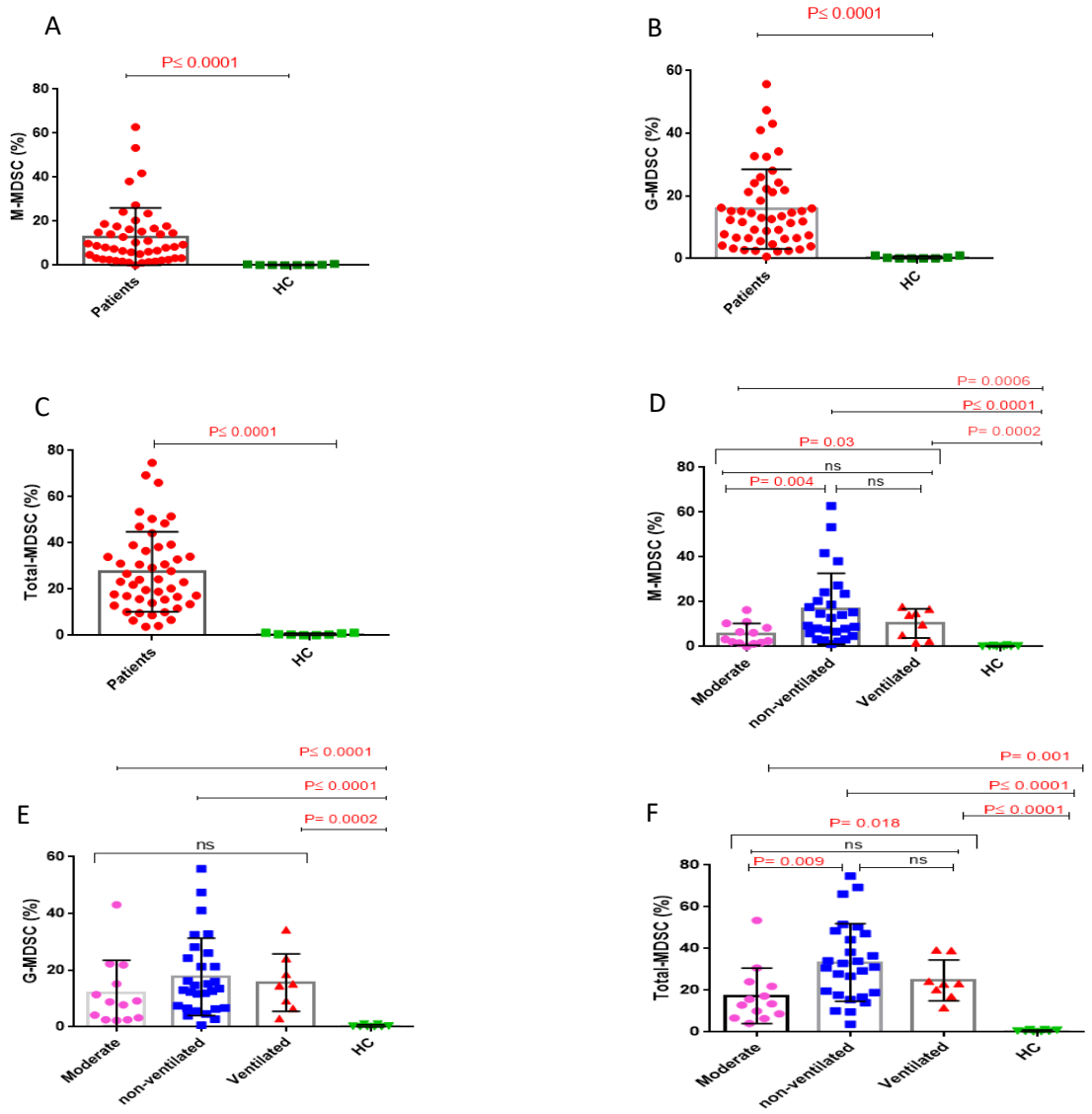


**Figure 2. Monocytic (M)-myeloid-derived suppressive cells (MDSCs) and granulocytic (G)-MDSCs frequency in COVID-19 patients and healthy subjects (HC).**

**Dot plot of the frequency of M-MDSCs (A), G-MDSCs (B), and Total-MDSC (C) between COVID-19 patients compared to HC without dividing the patients base on disease severity. D) Frequency of M-MDSCs individually was calculated based on disease severity. Individual values for each subject with the mean (SD) in severe COVID-19 patients admitted to the intensive care unit (ICU) who were either ventilated or non-ventilated or who had moderate disease and were not admitted to the ICU are presented as a dot plot.**

**E) Frequency of G-MDSCs individually was calculated based on disease severity. Individual values for each subject with the mean (SD) in severe COVID-19 patients admitted to the intensive care unit (ICU) who were either ventilated or non-ventilated or who had moderate disease and were not admitted to the ICU are presented as a dot plot. F) Frequency of Total-MDSCs individually was calculated based on disease severity. Individual values for each subject with the mean (SD) in severe COVID-19 patients admitted to the intensive care unit (ICU) who were either ventilated or non-ventilated or who had moderate disease and were not admitted to the ICU are presented as a dot plot. (⊢⊣ : shows significance between two groups by using t-test or Mann-Whitney U test, ⌐¬ : shows statistic statistical ventilated, non-ventilated, and moderate patients by using one-way analysis of variance).**

**Correlation between M-MDSCs/G-MDSCs/IL-8 with Clinical and Serum Parameters Outcome**

The relationships between M-MDSCs, G-MDSCs, total-MDSC frequency, and IL-8 concentration with serum biochemical markers LDH, ESR, CRP, troponin, CPK, CK-MB activity, and D-dimer, INR, age and arterial O2 saturation (PaO2FiO2) were evaluated. G-MDSC frequency correlated negatively with INR (r=-0.39, $p$=0.02), and the frequency of total-MDSC associated negatively with PaO2FiO2 %. (Table 4).

**Table 2. The frequency of myeloid-derived suppressive cells (MDSCs) and interleukin (IL)-8 levels in serum of COVID-19 patients and healthy controls**

| | COVID-19 Patients | HC | *p* value |
|---|---|---|---|
| **M-MDSC (%)** | 12.7±13.3<br>N=48 | 0.19±0.20<br>N=8 | **≤ 0.0001** |
| **G-MDSC (%)** | 15.8±12.6<br>N=50 | 0.35±0.40<br>N=8 | **≤ 0.0001** |
| **Total-MDSC (%)** | 27.5±17.3<br>N=48 | 0.55±0.41<br>N=8 | **≤ 0.0001** |
| **IL-8** | 6.4±7.8<br>N=32 | 0.10±0.0<br>N=8 | **0.03** |

Values were presented as Mean ± SD. $p$<0.05 was considered significant, HC; Healthy control, IL; interleukin, MDSC; myeloid-derived suppressive cell, G; granulocytic, M; monocytic.

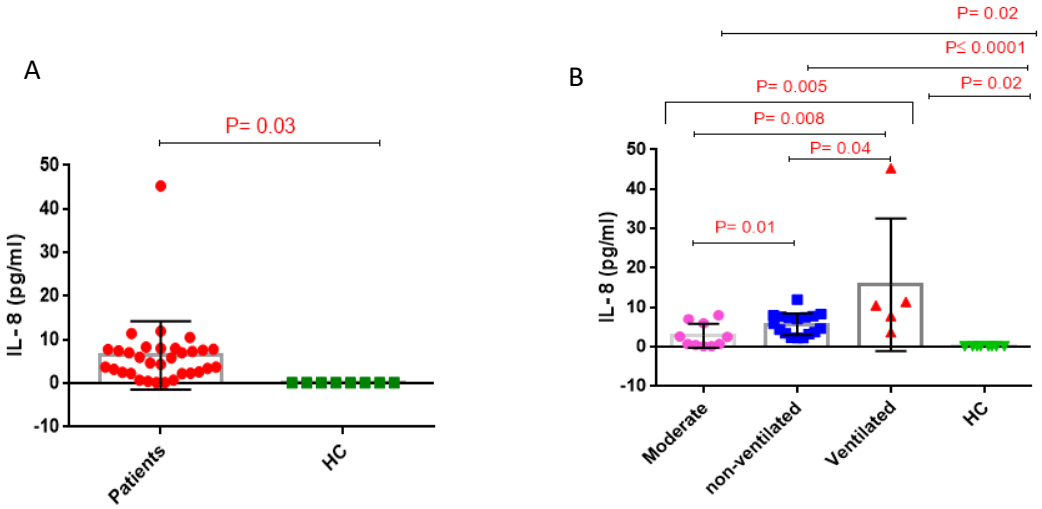


**Figure 3. Interleukin (IL)-8 levels in COVID-19 patients. Serum IL-8 concentration in COVID-19 patients versus healthy controls (HC) (A). Serum IL-8 levels in severe COVID-19 patients admitted to the intensive care unit (ICU) who were either ventilated or non-ventilated or had the moderate disease (B). Results are presented as dot plots of individual values for each subject with the mean (SD). (⊢⊣: shows significance between two groups by using t-test or Mann-Whitney U test, ⌐¬: shows statistic differences among ventilated, non-ventilated, and moderate patients by using one-way analysis of variance).**

**Table 3: The frequency of myeloid-derived suppressive cells (MDSCs) and serum interleukin (IL)-8 levels in severe COVID-19 patients according to intensive care unit (ICU) status**

| | HC | M (non-ICU) | S (ICU) | | *p* value | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | N=8 | | NV | V | M vs. HC | NV vs. HC | V vs. HC | S vs. M | NV vs. M | V vs. M | NV vs. V |
| **M-MDSC (%)** | 0.19±0.20 | 5.4±4.8 N=13 | 16.8±15.8 N=27 | 10.3±6.4 N=8 | 0.0006 | ≤0.0001 | 0.0002 | 0.03 | 0.004 | 0.06 | 0.43 |
| **G-MDSC (%)** | 0.35±0.40 | 11.8±11.6 N=13 | 17.6±13.6 N=29 | 15.6±10.0 N=8 | ≤0.0001 | ≤0.0001 | 0.0002 | 0.40 | 0.19 | 0.46 | 0.70 |
| **Total-MDSC (%)** | 0.55±0.41 | 17.3±13.3 N=13 | 33.3±18.5 N=27 | 24.7±9.8 N=8 | 0.001 | ≤0.0001 | ≤0.0001 | 0.018 | 0.009 | 0.19 | 0.22 |
| **IL-8** | 0.10±0.00 | 2.8±3.0 N=10 | 5.7±2.7 N=17 | 15.7±16.7 N=5 | 0.02 | ≤0.0001 | 0.02 | 0.005 | 0.01 | 0.008 | 0.04 |

Values were reported as Mean ± SD. $p<0.05$ was considered significant.

HC: Healthy Controls, M: Moderate, S: Severe, V: Ventilated, NV: Non-Ventilated.

MDSCs; Myeloid-derived suppressive cells, G; granulocytic, M; monocytic. IL; interleukin.

**Table 4. Correlation analysis between monocytic (M)-myeloid-derived suppressive cells (MDSC), granulocytic (G)-MDSC, interleukin (IL)-8, and other factors in COVID-19 patients**

| | ESR | | CRP | | LDH | | CPK | | Troponin | | CK-MB | | O2 sat% | | D-Dimer | | INR | | Age | | IL-8 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | r | p | r | p | r | p | r | p | r | p | r | p | r | p | r | p | r | p | r | p | r | p |
| **M-MDSC** | 0.01 | 0.95 | 0.004 | 0.98 | -0.04 | 0.75 | -0.10 | 0.52 | -0.10 | 0.59 | -0.12 | 0.58 | -0.30 | 0.12 | -0.26 | 0.21 | -0.12 | 0.53 | 0.12 | 0.40 | 0.03 | 0.85 |
| **G-MDSC** | 0.05 | 0.74 | 0.12 | 0.51 | 0.14 | 0.33 | -0.09 | 0.55 | -0.20 | 0.29 | -0.31 | 0.12 | -0.10 | 0.58 | -0.27 | 0.17 | -0.39 | **0.02** | -0.12 | 0.39 | -0.03 | 0.84 |
| **Total-MDSC** | 0.007 | 0.96 | 0.146 | 0.45 | 0.07 | 0.65 | -0.19 | 0.22 | -0.22 | 0.26 | -0.29 | 0.17 | -0.39 | 0.04 | -0.36 | 0.07 | -0.32 | 0.08 | 0.21 | 0.11 | 0.17 | 0.30 |
| **IL-8** | 0.008 | 0.96 | -0.23 | 0.27 | 0.14 | 0.45 | 0.10 | 0.57 | 0.11 | 0.60 | 0.22 | 0.28 | 0.17 | 0.41 | -0.26 | 0.27 | -0.15 | 0.46 | 0.25 | 0.16 | - | - |

r: Pearson correlation coefficient, p: *p*-value

CK-MB: creatinine kinase-muscle/brain; CPK: creatine phosphokinase CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; INR: international normalized ratio; LDH: lactate dehydrogenase: O2 sat %; percentage oxygen saturation.

## DISCUSSION

We show here that the frequencies of M-MDSCs and G-MDSCs in the systemic circulation increased in Iranian COVID-19 patients compared to the rates observed in healthy subjects. M-MDSC frequency was significantly elevated in non-ventilated severe COVID-19 patients compared to patients with moderate COVID-19 disease. In addition, IL-8 levels in the serum of COVID-19 patients were significantly higher than in healthy control subjects and ventilated patients with severe COVID-19 compared to severe COVID-9 patients who were not ventilated.

MDSCs are immature myeloid cells that can down-regulate T cell responses[13]. CXCR1 and CXCR2, the receptors for IL-8, are expressed on M-MDSCs and G-MDSC; suggesting that IL-8 may recruit MDSCs from the systemic circulation to the local tumor environment.[10] Elevated levels of serum IL-8 are also linked to COVID-19 severity.[25] However, circulating IL-8 and MDSCs were reported to be similar in COVID-19 patients in ICU compared to those not in ICU in a

single study.[26] COVID-19 patients presenting with severe respiratory failure have a reduced level of HLA-DR on CD14 monocytes.[27] while the presence of CD14+ HLA-DR$^{low}$ cells was associated with immune dysregulation in patients with severe COVID-19.[27] The systemic expansion of MDSC populations may account for the dysregulated immune function of T-cells, particularly CD8 T-cells, reported in SARS-CoV-2 infected patients who develop ARDS.[28]

A more significant percentage of G-MDSCs has been reported in severe and mild COVID-19 patients than in healthy subjects.[25] This contrasted with the decreased numbers of systemic MDSCs in convalescent severe COVID-19 patients which correlated with elevated circulating blood levels of IL-8, IL-1β, and TNF-α and reduced blood levels of TGF-β.[3,25,29] COVID-19 disease duration is associated positively with circulating levels of the neutrophil chemoattractant IL-8, which may account for the neutrophil/lymphocyte ratio (NLR) being an excellent SARS-COV-2 infection marker.[30]

This report shows an elevated frequency of circulating G-MDSC cells in patients with either moderate or severe COVID-19 compared to the levels in healthy subjects. However, no significant differences existed between patients with moderate or severe disease. The frequency of G-MDSC in blood taken at admission to the hospital was raised in Italian patients with severe COVID-19 who died compared to the levels seen in survivors.[31] In addition, there were higher levels of plasma IL-8 in non-survivors compared with survivors upon admission, although the IL-8 levels in non-survivors declined to comparable levels to survivors over time.[31] Other reports support our data showing higher serum IL-8 levels in COVID-19 patients who were ventilated.[31] It is difficult to explain the differences in G-MDSC cell frequency between our study and the earlier study from Italy but postulate that changes in treatment between the studies, ethnic differences, or the study numbers. In summary, the results presented here fail to support the hypothesis that the numbers of MDSCs in peripheral blood predict the clinical outcomes of COVID-19.

We report differences in peripheral blood levels of IL-8 between severe COVID-19 patients depending on whether they were ventilated or non-ventilated and that patients with no disease or moderate disease have significantly lower peripheral blood IL-8 levels than subjects with severe disease. We also failed to report any differences in G-MDSC frequency in the peripheral blood according to COVID-19 severity, although peripheral blood IL-8 levels were elevated in COVID-19 patients. However, peripheral blood M-MDSC frequencies were greater in non-ventilated severe COVID-19 patients than in patients with moderate COVID-19.

There was no correlation between peripheral blood levels of IL-8 and MDSC frequencies. This indicates that either other factors drive MDSCs generation in these patients or that elevated blood IL-8 levels indicate much more significant levels in the airways and lung or other COVID-19-infected tissues and that this causes tissue margination. This effect may be more important in very severe COVID-19 who are ventilated where M-MDSC margination into the lung or kidney could cause local immune suppression. Future studies using matched lung samples and circulating blood may resolve this issue since the frequency of M-MDSC cells is elevated in the peripheral blood of patients with COVID-19 but unchanged in the upper airways.[17]

MDSCs expansion in patients with HIV-1 promotes regulatory T-cell differentiation and the modulation of T-helper cell functions.[32] MDSCs counter viral persistence in patients with HIV-1 and suggests a potential role in COVID-19 and other virus-evoked diseases.[32] In contrast, adverse outcomes of septic shock are significantly linked with raised M-MDSCs levels.[33] In sepsis, MDSCs have a dual role[34] by attenuating inflammation in the early stages of the disease and evoking long-term immunosuppression in late-stage condition.[25,34]

Overall, the data presented here for Iranian COVID-19 patients corresponds well with other studies demonstrating that elevated peripheral blood IL-8 levels correlated with worse clinical outcomes. We did not see a relationship between blood IL-8 concentrations and systemic MDSC frequencies, suggesting that other factors may modulate the maturation of systemic MDSCs or that factors localized to the airways and lungs play a more significant role in the recruitment and expansion of MDSCs. This cytokine-MDSC network makes a novel target to improve the clinical outcomes in patients with severe or very severe COVID-19. Further studies are needed comparing lung and peripheral blood cell frequencies with a much more comprehensive range of pro-inflammatory mediators.

## CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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