Immunological predictors of disease severity in patients with COVID-19 infection

Asma Al Balushi[1], Jalila Al Shekaili[2], Mahmood Al Kindi[2], Zainab Ansari[3], Murtadha Al Khabori[2], Faryal Khamis[3], Zaiyana Ambusaidi[3], Afra Al Balushi[3], Aisha Al Huraizi[3], Sumaiya Al Sulaimi[3], Fatma Al Fahdi[3], Iman Al Balushi[2], Nenad Pandak[3], Tom Fletcher[4], Eskild Petersen[5], and Iman Nasr[3]

[1]Sohar Hospital
[2]Sultan Qaboos University Hospital
[3]The Royal Hospital
[4]Royal Liverpool University Hospital
[5]Aarhus University Hospital

March 07, 2024

Abstract

Background: Identifying immune cells involved in COVID-19 disease progression and predictors of poor outcomes is important to manage patients adequately. Methods: A prospective observational cohort study enrolled 53 mild non-hospitalized and 48 hospitalized confirmed COVID-19 patients to a tertiary hospital in Oman. Results: Hospitalized patients were older (58 years vs 36 years, p <0.001) and had more comorbid conditions like diabetes (65 % Vs 21% p<0.001). Hospitalized patients had significantly higher inflammatory markers (p<0.001); C-reactive protein (CRP) (114 vs 4 mg/L), Interleukin-6 (IL-6) (33 vs 3.71pg/ml), lactate dehydrogenase (LDH) (417 vs 214 U/L), ferritin (760 vs 196 ng/mL), fibrinogen (6 vs 3 g/L), D-dimer (1.0 vs 0.3 mcg/mL), disseminated intravascular coagulopathy (DIC) score (2 vs 0) and neutrophil/lymphocyte ratio (4 vs 1.1), (p<0.001). In multivariate regression analysis, statistically significant independent early predictors of ICU admission or death were higher levels of IL-6 (OR 1.03, p=0.03), frequency of large inflammatory monocytes (CD14+CD16+) (OR 1.117, p=0.010) and frequency of circulating naïve CD4+ T cells (CD27+CD28+CD45RA+CCR7+) (OR 0.476, p=0.03). Conclusion: IL-6, frequency of large inflammatory monocytes, and circulating naïve CD4 T cells can be used as independent immunological predictors of poor outcomes in COVID-19 patients to prioritize critical care and resources.

Immunological predictors of disease severity in patients with COVID-19 infection

Asma Al Balushi, Jalila AlShekaili, Mahmood Al Kindi, Zainab AL Ansari, Murtadha Al-Khabori, Faryal Khamis, Zaiyana Ambusaidi, Afra Al Balushi, Aisha Al Huraizi, Sumaiya Al Sulaimi, Fatma Al Fahdi, Iman Al Balushi, Nenad Pandak, Tom Fletcher, Eskild Petersen, Iman Nasr

Authors names, affiliations, address, emails and contributions:

1. Asma Al Balushi (BSc, MD, OMSB, MRCP UK, DTMH, FRCPath, DipUKMP): Conceptualized, designed the study, wrote the research proposal, data collection for inpatients and field visits, communicated with patients, drafted and reviewed the manuscript, and overall workflow and integrity supervision. Address: Tropical and Infectious Diseases Unit, The Royal Liverpool University Hospital, Prescot street, Liverpool L7 8XP, United Kingdom. Present address: Internal Medicine department, Suhar hospital, Suhar, Oman. P. O. Box 49, Muwailih 311. Email: thebrightsoul2@gmail.com

2. Jalila Al shekaili (BSc, MD, FRCPA, Ph.D.): Reviewed study design, reviewed research proposal, supervised receiving blood samples at the immunology lab, supervised the flow cytometry work and IL-6 assessment run and analysis, and interpreted the data, wrote up the first few drafts and approved the final draft of the manuscript. Address: Department of Microbiology and Immunology, Sultan Qaboos University Hospital, Seeb, Oman. P. O. Box 38, Alkhoudh 123. Email: jalila@squ.edu.om
3. Mahmood Al Kindi (BSc, MD, FRCPA, Ph.D.): Reviewed study design, reviewed research proposal, supervised receiving blood samples at the immunology lab, supervised the flow cytometry work and IL-6 assessment run and analysis, analyzed the data, wrote up the first few drafts, and approved the final draft of the manuscript. Address: Department of Microbiology and Immunology, Sultan Qaboos University Hospital, Seeb, Oman. P. O. Box 38, Alkhoudh 123. Email: dralkindi@squ.edu.om
4. Zainab Al Ansari (MD): Field visits, data, and sample collection from outpatients and inpatients and data entry. Address: Internal Medicine department, Royal Hospital, Muscat, Oman. P.O. Box 1331, Al Ghubra area 111. Email: zainabahameed99@gmail.com
5. Murtadha Al-Khabori, (MD): Data analysis, writing up results, and manuscript review. Address: Clinical Haematology Department, Sultan Qaboos University, Muscat, Oman. P. O. Box 35, Alkhoudh 123. Email: khabori@squ.edu.om
6. Faryal Khamis, (MD): Manuscript review and facilitating research work for inpatients. Address: Internal Medicine department, Royal Hospital, Muscat, Oman. P.O. Box 1331, Al Ghubra area 111. Email: khami001@gmail.com
7. Zaiyana Ambusaidi (MD): Data and sample collection from inpatients and data entry. Address: Internal Medicine department, Royal Hospital, Muscat, Oman. P. O. Box 1331, Al Ghubra area 111. Email: z_ambusaidi@hotmail.com
8. Afra Al Balushi (MD, OMSB Internal medicine): Data and sample collection from inpatients and data entry. Address: Internal Medicine department, Royal Hospital, Muscat, Oman. P.O. Box 1331, Al Ghubra area 111. Email: afraa.albalushi@gmail.com
9. Aisha Al Huraizi (MD, OMSB Internal medicine): Data and sample collection from inpatients and data entry. Address: Internal Medicine department, Royal Hospital, Muscat, Oman. P.O. Box 1331, Al Ghubra area 111. Email: aisha.huraizi@gmail.com
10. Sumaiya AL Sulaimi (MD, OMSB Internal medicine): Data and sample collection from inpatients and data entry. Address: Internal Medicine department, Royal Hospital, Muscat, Oman. P.O. Box 1331, Al Ghubra area 111. Email: samasoomi@hotmail.com
11. Fatma Al Fahdi (MD, OMSB Internal medicine): Data and sample collection from inpatients and data entry. Address: Internal Medicine department, Royal Hospital, Muscat, Oman. P.O. Box 1331, Al Ghubra area 111. Email: alfahdi56370@yahoo.com
12. Iman Al Balushi (MD): Data collection and entry for outpatients and second review of all data. Address: Sultan Qaboos University Hospital, Muscat, Oman. P. O. Box 35, Alkhoudh 123. Email: emanalbeloshi@gmail.com
13. Nenad Pandak (MD, Ph.D.): Manuscript review and facilitating research work for inpatients. Address: Internal Medicine department, Royal Hospital, Muscat, Oman. P.O. Box 1331, Al Ghubra area 111. Email: npandak@gmail.com
14. Tom Fletcher (MD, Ph.D.): Overall supervision of the study and manuscript review. Address: Tropical and Infectious Diseases Unit, Royal Liverpool University Hospital, Prescot St, Liverpool L7 8XP, United Kingdom. Email: tom.fletcher@lstmed.ac.uk
15. Professor Eskild Petersen. Helped in conceptualizing research idea and manuscript review. Aarhus University Hospital Institute of Clinical Medicine, Department of Infectious Diseases, Palle Juul-Jensens Boulevard 45, 8200, Aarhus N, Denmark. Email: eskild.petersen@clin.au.dk and eskild.petersen@gmail.com
16. Iman Nasr (MD): Proposal review, registered the study, communication with laboratories and companies, manuscript review, and overall workflow and integrity supervision. Address: Internal Medicine department, Royal Hospital, Muscat, Oman. P.O. Box 1331, Al Ghubra area 111. Email: drimannasr@gmail.com

Corresponding author: Iman Nasr (MD). Address: Internal Medicine department, Royal Hospital, Muscat, Oman. P.O. Box 1331, Al Ghubra area 111. Mobile: (+968) 99832007Email: drimannasr@gmail.com**Alternate corresponding author:** Asma Al Balushi (BSc, MD, OMSB, MRCP UK, DTMH, FRCPath, DipUKMP). Address: Internal Medicine department, Suhar hospital, Suhar, Oman. P. O. Box 49, Muwailih 311. Mobile number: (+968) 92319222. Email: thebrightsoul2@gmail.com

Funding: The study was supported by the Center of Studies and Research in Oman (TRC/CRP/MOH/COVID-19/20/26).

Conflict of interest: The authors declare that they have no financial interests or personal relationships that might influence the work presented in this paper.

Ethical approval:

Ethical approval was obtained through the Central Research Committee at the Ministry of Health in Oman (MoH/CSR/20/23605).

Consent:

Informed consent was obtained from all enrolled patients.

Highlights:

- COVID-19 severity predictors help to prioritize resources and minimize mortality
- Immunological markers can be used as predictors of poor prognosis
- High IL-6 independently predicts poor outcomes in COVID-19 patients
- High percentage of large inflammatory monocytes predicts worse COVID-19 outcomes
- Lower circulating Naïve CD4+ T cells predicts poor prognosis in COVID-19 patients

Abstract:

Background: Identifying immune cells involved in COVID-19 disease progression and predictors of poor outcomes is important to manage patients adequately.

Methods: A prospective observational cohort study enrolled 53 mild non-hospitalized and 48 hospitalized confirmed COVID-19 patients to a tertiary hospital in Oman.

Results: Hospitalized patients were older (58 years vs 36 years, p <0.001) and had more comorbid conditions like diabetes (65 % Vs 21% p <0.001). Hospitalized patients had significantly higher inflammatory markers (p <0.001); C-reactive protein (CRP) (114 vs 4 mg/L), Interleukin-6 (IL-6) (33 vs 3.71pg/ml), lactate dehydrogenase (LDH) (417 vs 214 U/L), ferritin (760 vs 196 ng/mL), fibrinogen (6 vs 3 g/L), D-dimer (1.0 vs 0.3 mcg/mL), disseminated intravascular coagulopathy (DIC) score (2 vs 0) and neutrophil/lymphocyte ratio (4 vs 1.1), (p <0.001). In multivariate regression analysis, statistically significant independent early predictors of ICU admission or death were higher levels of IL-6 (OR 1.03, p =0.03), frequency of large inflammatory monocytes (CD14+CD16+) (OR 1.117, p =0.010) and frequency of circulating naïve CD4+ T cells (CD27+CD28+CD45RA+CCR7+) (OR 0.476, p =0.03).

Conclusion: IL-6, frequency of large inflammatory monocytes, and circulating naïve CD4 T cells can be used as independent immunological predictors of poor outcomes in COVID-19 patients to prioritize critical care and resources.

Keywords:

COVID-19, lymphocyte subsets, Inflammatory markers, Immunological predictors, mortality predictors

Introduction:

A cluster of atypical viral pneumonia cases was identified in Wuhan, China, in December 2019. A novel coronavirus has been identified as the cause, named later as Severe Acute Respiratory Syndrome -2 (SARS-CoV-2)[1, 2]. The World Health Organization (WHO) declared it a pandemic on 11 March 2020, with

total confirmed cases exceeding 121 million cases worldwide and over 2.6 million deaths by 20th March 2021 [3]. Some people develop severe coronaviruses disease 2019 (COVID-19) disease while others remain asymptomatic or have a milder illness course [4]. Identifying predictors of poor outcomes is increasingly gaining importance to help to prioritize resources for high-risk patients and minimizing death. Older age and certain comorbid conditions like chronic renal, lung, and heart diseases are established predictors of worse prognosis in COVID-19 patients. In addition, hypoxemia, diarrhea, and high inflammatory markers like C-reactive protein (CRP) and interleukin-6 (IL-6) on admission are other predictors of worse prognosis [4-7].

In these patients, immune cells, namely lymphocytes, have been heavily implicated in controlling disease progression and clinical outcomes. Some studies have demonstrated that high leukocytes, specifically neutrophils [4, 7, 8], and T cell lymphopenia (CD3+, CD8+ [9, 10] and CD4) [11] are associated with increased mortality in patients admitted with COVID-19 pneumonia. Moreover, it was shown that older patients have lower counts and frequency of naïve (CD45RA+CCR7+CD27+CD28+) CD4+T cell contributing to the poor response of T cells [12]. These cells are required for the effective handling of new infections or vaccines [13, 14]. It has been already shown that hospitalized COVID-19 patients have reduced (CD45RA+CCR7+CD27+CD28+) CD4+ naïve subsets of T cells compared to healthy uninfected controls [15]. Furthermore, hospitalized patients with severe manifestations have a lower frequency of exhausted non-cytotoxic T cells (PD-1+ CD57-CD8+) [16].

Monocytes are other immune cells that are vital for normal and dysregulated immune response. Monocytopenia was found to be a predictor of worse outcomes in patients with severe community infections and sepsis [17]. Moreover, there is a reduction in the classic monocytes (CD14+CD16-) in severe COVID-19 infection and an increase in the inflammatory subsets (CD14+CD16+) [18]. Monocytes were also recently divided based on size into small and large subsets, coupled with a level of CD14 and CD16 expression into different subsets with different functional abilities[19].

Our study aimed to identify changes in immune variables, namely naïve CD4+ and CD8+, (CD45RA+CCR7+ CD27+CD28+), exhausted T cells CD8+ (PD-1+ CD57-), large and small inflammatory (CD14+CD16+) monocytes, and IL-6 level early in the course of COVID -19 infection as immunological predictors of poor outcomes including ICU admission and death.

Methods:

Study design and patients

This was a prospective observational cohort study conducted from 20 July 2020 to 27 August 2020. A total of 101 confirmed COVID-19 cases by SARS-CoV-2 real-time polymerase chain reaction (PCR) from nasopharyngeal swabs were enrolled (53 non-hospitalized and 48 hospitalized cases). Inclusion criteria were: patients aged 13 years or older, both genders, confirmed mild COVID-19 infection in the non-hospitalized group, and confirmed moderate infection in patients hospitalized at the Royal Hospital (RH). Hospitalized patients were recruited within 48 hours of admission. Patients admitted directly to the ICU at the time of enrolment were excluded from the study. Mild cases were those who did not require admission to the hospital due to COVID-19 related illness or oxygen therapy. In contrast, moderate cases were identified as patients with hypoxemia </= 94% requiring oxygen support or those with one or more COVID-19 related organ involvement.

A specified clinical team was assigned to collect data and blood samples from the inpatients. Outpatients were approached through a daily list of confirmed COVID-19 patients provided by the Center of Operation Management for COVID-19 at the Ministry of Health, Oman. Telephonic calls were conducted to get patients' consent to participate in the study after an explanation of the research idea. Patients in the community were visited by two designated researchers the next day. One nominated researcher-maintained communication to ensure the adherence of participants. Patients' demographics and clinical characteristics were obtained from non-hospitalized patients directly or for hospitalized ones through electronic hospital records using a unified data collection form. Informed consent was obtained from all enrolled patients.

Measurement of inflammatory markers and lymphocyte subsets

Blood was collected on median days of 6 (2-8) for the hospitalized patients and 7.5 (6.75-8.25) for the non-hospitalized group from the onset of symptoms. Blood was sent for complete blood count (CBC), renal function tests (RFT), liver function tests (LFT), lactate dehydrogenase (LDH), ferritin, D-dimer, coagulation profile, IL-6, and lymphocyte subsets analysis. IL-6 concentration was measured on serum samples using fully automated Elecsys IL-6 immunoassay (electrochemiluminescence immunoassay) on Cobas e 601 immunoassay analyzers (Roche Diagnostics, Switzerland) following the manufacturer's protocol. The used cut-off of IL-6 was 7.0 pg/ml.

Assessment of different basic lymphocyte and detailed T cell subsets using flow cytometry was performed. DuraClone IM T cell subsets tube (Beckman coulter) that includes CD45RA (clone, 2H4), CD197 (CCR7) (clone, G043H7), CD28 (clone, CD28.2), CD279 (PD1) (clone, PD1.3.5), CD27(clone, 1A4.CD27), CD4(clone, 13B8.2), CD8(clone, B9.11), CD3(clone, UCHT-1), CD57(clone, NC1), CD45(clone, J33) was used to assess different T cell subsets. DuraClone IM phenotyping basic tube (Beckman coulter) that includes: CD16(3G8), CD56(N901), CD19(J3_119), CD14(RMO52), CD4 (13B8.2), CD8(B9.11), CD3(UCHT-1), CD45(J33) was used for basic lymphocyte staining. Gating strategies are presented in figure1 and 2. A total of 100,000 event were collected.

Briefly, 100 µl of blood was added to the tube containing the desired cocktail of antibodies and incubated for 20 minutes at room temperature. 100 µl of lysing solution optilyse-B or Versalyse was added according to the manufacture's recommendation. This was followed up with a wash step. Acquisition of samples was done using Navios flow cytometry (Beckman coulter) and analyzed using Kaluza version 2.1(Beckman coulter).

Statistical analysis

Results were expressed as medians with their interquartile ranges (IQR) and frequency (%) for continuous and categorical variables, respectively. Assessment of differences between inpatients and outpatients was performed using Chi-squared test, or Fisher's exact test for categorical variables, and Mann-Whitney U test for continuous variables.

In addition, selected variables from the demographic, clinical presentation, inflammatory markers, and all significant immune-subsets were subjected to a univariable logistic regression with the composite outcome of ICU admission and mortality in the first 30 days. Variables significant in the univariable analyses (p <0.05) were assessed using multivariable logistic regression to determine the independent predictors of the mentioned outcome.

An alpha threshold of 0.05 was used for statistical significance. Statistical analysis was performed using R studio (RStudio Team (2016). RStudio (Version 1.1.456): Integrated Development for R. RStudio, Inc., Boston, MA URL).

Results:

Demographic data, clinical characteristics, and main laboratory findings

During the period between July 2020 and August 2020, a total of 53 mild non-hospitalized and 48 moderate hospitalized patients with COVID-19 infection were recruited. Demographic, clinical characteristics, and laboratory investigations, including immunological and inflammatory biomarkers, were compared between the two groups.

Hospitalized patients were found to be older (58 years *vs.* 36 years, $p < 0.001$). On the other hand, there was no difference in gender distribution (54% of hospitalized *vs.* 43% of the non-hospitalized were male, p =0.32). Comorbid conditions were more frequent in the admitted group, such as diabetes (65 %*vs.* 21%, p <0.001) and those on oral hypoglycemic agents/insulin (40 % *vs.* 6%, $p < 0.001$).). While shortness of breath was more frequent in hospitalized patients (80 % *vs.* 17%, p <0.001), fatigue (56.6% *vs.* 20.83%, p <0.001) and diarrhea (34.0% *vs.* 14.58%, p=0.037) were reported more in the non-hospitalized group (Table1).

Hospitalized group had higher inflammatory markers: CRP (114 *vs.*4 mg/L, p <0.001), LDH (417 *vs.* 214 U/L,p <0.001), ferritin (760 *vs.* 196 ng/mL,p <0.001), fibrinogen (6 *vs.* 3 g/L,p <0.001), D-dimer (1.0 *vs.* 0.3 mcg/mL,p <0.001), disseminated intravascular coagulopathy (DIC) score (2.0 *vs.* 0.0, p <0.001) and higher neutrophil/lymphocyte ratio (4 *vs.* 1.1, p <0.001) as shown in Table 2.

Immunological features

CD3 lymphopenia in the hospitalized group

Despite having higher total white blood cells and neutrophils (Table 2), hospitalized patients had a lower lymphocyte count and a lower percentage of CD3+ T cell subset (p =0.001). At the same time, the ratio of CD4+T cells and CD8+T cells were normal (Table 3), and there was no difference between the two groups when comparing the percentage of CD19+ B cells and CD16+56+ NK cells (p =0.29 and 0.42 respectively) (Table 3).

Reduced naïve and increased effector and increased cytotoxic and exhausted CD4+T cells in the hospitalized group

Assessment of CD4+ T cells maturation and differentiation stages has revealed a significantly higher increase in the total naïve (CD45RA+CCR7+) CD4+T cells in the non-hospitalized group with a median of 32.75% (IQR 23.51-39.50) compared to 5.40% (14.99-36.88) -39.50) in the hospitalized group (p = 0.034). This increase was mirrored by the increase in the naïve (CD27+CD28+ CD45RA+CCR7+) CD4+ T cells subsets with a median of 99.48% (IQR98.90-99.71) *vs.* 99.00% (IQR 97.15-99.67) in the non-hospitalized group compared to the admitted group respectively (p = 0.021) (Table 3). On the other hand, there was an expansion of effector CD4+T cells in hospitalized patients compared to non-hospitalized patients. These patients had a higher frequency of exhausted (PD-1+CD57-) CD4+ T cells with a median of 17.59 % (IQR 14.16-23.48) *vs* .13.63% (IQR 10.24-18.20), (p =0.001) and exhausted cytotoxic (PD1+CD57+CD4+) CD4+ T cells with a median of 5.48% (IQR 1.66-12.89) compared to 2.97% (IQR 1.56-5.98) the non-hospitalized patients (p =0.039) (Table 3).

Reduced naïve and increased cytotoxic effector and exhausted CD8+T cells in the hospitalized group

Similar to total CD4+T cells, there was no statistically significant difference in total CD8+T cells (p = 0.317). However, there was an increase in the naïve CD8+T cells seen in the non-hospitalized patients with a median of 27.82% 27.82 (IQR 14.84-37.89) compared to14.31 (IQR 4.485-36.240) in the hospitalized group (p = 0.010). In addition, non-hospitalized group had higher frequency of cells with lower cytotoxic characteristics. Examples include effector memory (TEM) CD27+CD28+ CD8+ T cell 43.95% [IQR 32.92-59.75] *vs* . (28.71 [IQR 21.38-45.66], p = 0.01) and revertant effector memory (TEMRA) CD27+CD28- CD8+ T cells 21.07% [IQR 15.00-28.39] *vs.*16.33% [IQR 10.29-27.73], p = 0.029), in the non-hospitalized*vs.* hospitalized group (Table 3).

In contrast, hospitalized group had a high percentage of cytotoxic TEM CD27-CD28- CD8+T cells with a median 47.25% (IQR 24.14-57.68) compared to 25.76% (IQR 15.45-40.84) in the non-hospitalized group (p = 0.002). Moreover, percentage of cytotoxic exhausted (CD57+PD-1+) CD8+ T cells were higher in the hospitalized group with a median of 16.86% (IQR 11.35-27.96) *vs* . 11.98% (IQR 8.96-15.41), p = 0.008) (Table 3). Hospitalized group had higher frequency of cells with cytotoxic characteristics.

Large inflammatory (CD14+ CD16+) monocytes in the admitted group

Hospitalized patients exhibited a lower percentage of CD14+ monocytes than non-hospitalized patients with a median of 3.91% (2.20-6.310) Vs. 7.43 (IQR 6.09-10.10), respectively, (p <0.001). As majority of the monocytes were of small size, the hospitalized group had a lower median of 2.46% (IQR 1.21-4.10) CD14+ small monocytes compared to the non-hospitalized grou with a median of 4.58% (IQR 3.34-5.53),p < 0.001), (Table 3).

Although there was no difference in the total large CD14+ monocytes, large inflammatory (CD14+ CD16+)

monocytes were seen at a higher percentage in the hospitalized group with a median of 27.3% (IQR 12.31-40.62) compared to those non-hospitalized with 15.29 % (IQR 11.39- 9.54), p <0.001), (Table 3).

Higher IL-6 levels in the hospitalized group

In line with previous findings, IL-6 was higher in the hospitalized disease group with a median of 33 pg/mL (IQR 8.36-86.28) compared to the non-hospitalized group 3.71 (IQR 1.58-12.580) (p <0.001), (Table 3).

ICU admission and death

Ten out of 48 (21%) hospitalized patients required ICU admission. A total of 7 patients died, five from those who were shifted to the ICU, one of them died while in the ward, and one from the non-hospitalized patients who died before 30 days of illness. Therefore, the total composite endpoint was 12 events (2%).

Factors associated with ICU admission or death

Univariable regression analysis was used to examine potential parameters predictive of ICU admission or death within 30 days, (Table 4). Significant univariable factors were then subjected to multivariable regression analysis. This has shown that the increase in IL-6 level (pg/mL) increases the composite endpoint's odds by 1.03 (p =0.03). Similarly, an increase in the percentage of large inflammatory monocytes (CD16+CD14+) subset is associated with an increase in the composite endpoint's odds by 1.117 (p =0.01). On the other hand, an increase in the frequency of the naïve CD4+ (CD45RA+CCR7+CD27+CD28+) decreases the odds of the composite endpoint by 0.476 (p =0.03), (Table 4).

Discussion:

Earlier studies have shown that in hospitalized patients, inflammatory biomarkers such as CRP, ferritin, LDH, d-dimer, and IL-6 can be used to predict clinical outcomes in COVID-19 patients[4-7]. The immune system plays a significant role in the disease's clinical manifestation and progression, including the above-mentioned inflammatory markers. Therefore, the focus on immunological predictors that can be used early in the disease course to enable relocation of resources toward those at risk of getting the severe disease should be prioritized. In this study, we have shown that, in addition to elevated level of IL-6, the higher percentage of large inflammatory CD14+ CD16+ monocytes and lower percentage of naive CD27+CD28+ CD4+T cells are independent early immunological prognostic predictors of worse outcome in patients with COVID-19.

Similar to other existing data, in the present study, admitted patients were found to be older, diabetic, and hypertensive compared to those who did not require admission. Moreover, underlying heart diseases, chronic lung diseases, and chronic renal failure were noted more often in admitted patients than non-admitted patients, which is in agreement with previous studies [20-23]. In addition, current literature has ample information suggesting that high inflammatory markers can be used as a predictor of worse outcomes in admitted patients with COVID-19 infection. These included white blood cell count (WBC), absolute lymphocyte count, LDH, CRP, procalcitonin, D-dimer, ferritin, and ESR [20, 24]. This was also confirmed in our study, in which admitted patients had higher inflammatory markers including CRP, LDH, ferritin, fibrinogen, D-dimer, DIC score, and higher neutrophil/lymphocyte ratio.

The immune CD4+ and CD8+ T cells can be divided into four main subsets based on the surface expression of CCR7 and CD45RA. They reflect different maturation and T-cell differentiation stages that are functionally distinct. The subsets included naïve CD4+T cell subsets (CCR7+CD45RA+), central memory (TCM) CD4+T cells (CCR7+CD45RA-), effector memory CD4+T cells (TEM) and RA+ revertant effector memory (CCR-CD45RA+) (TEMRA)[25, 26]. Both CD4+ and CD8+ main four subsets can be further divided into different functional subsets based on the expression of CD27 and CD28 with different cytokine expression [12, 26, 27]. TEM that are CD27-CD28- are mainly IFN-g producers (Th1) compared to the CD27-CD28+TEM that are IL-2(Th0), INF-g(Th1), and IL-4 (Th2) producers[25]. TEM and TEMRA T cells are good cytokine producers, including IL-2, IFN-g, and TNF-a. Moreover, CD27-CD28- T cells have high effector capability similar to the terminal effector T cells TEMRA subset -[12, 26]. Similarly, the combination of PD-1 and CD57 can identify cells with exhausted and or cytotoxic phenotype[28, 29].

On the other hand, naïve T cell is mainly CD27+CD28+, and it is a good producer of IL-2 that is required for activation and proliferation [12, 25]. CD27+CD28+ naïve T cells are crucial in response to a new virus or vaccine. Those with reduced frequency of naïve T cells, as in the elderly, are at risk of getting a significant disease compared to those with plenty of naïve T cells that can respond better to such new viruses [30]. This was one of the important explanations for the increased mortality in the elderly after infection with SARS-CoV-2. This is in line with our findings that those with a low percentage of naïve CD4+T cells are at a higher risk of increased mortality.

De Biasi has compared the immune system in mild to moderate hospitalized patients(n=39), versus healthy uninfected group (n=25). This has shown a low count of total CD4+ and CD8+ T cells and their naïve and TCM subsets in the patient's group. Moreover, these patients had a higher frequency of cells with senescent/exhausted phenotype (CD57+PD-1+) [15]. Similarly, we have found reduced percentage of naïve CD4+T and CD8+T and increased exhausted CD4+ and CD8+T cells in the hospitalized group early in the disease course. Studying patients with a milder illness almost three weeks from infection, non-admitted(n=17) compared to the admitted ones (n=13 moderate and n=9 severe), has shown a higher percentage of exhausted CD8+ T cells with a lesser cytotoxicity and inflammatory profile than the patients with severe manifestations [16]. We have, as well, showing that hospitalized group had increased percentage of exhausted and cytotoxic T-cell phenotype in the hospitalized group.

Monocytes are the key immune cells and good producers of inflammatory cytokines like IL-6 [31, 32]. They acquire a bigger size upon activation and viral infections [33], including severe COVID-19 [34]. Moreover, monocytes can be divided according to the differentiation stage using a combination of CD markers into (CD14+CD16-) immature, differentiated and inflammatory type (CD14+CD16+) and non-classical (CD14-CD16+) [35]. Examining monocytes in this way, has revealed higher percentage of large inflammatory monocytes CD14+ CD16+ detected in the hospitalized, in line with previous suggestions that patients with severe manifestation have bigger sized monocytes [33, 34, 36].

Conclusion:

The current study identifies elevated level of IL-6, higher percentage of CD14+CD16+ inflammatory large monocytes and lower percentage of circulating naïve (CD27+CD28+CD45RA+CCR7+) CD4+ T cells early in the disease course as independent early predictors of ICU admission or death in patients with COVID-19 infection. Such predictors can be used for early identification of patients who might deteriorate and thus need early aggressive interventions. Larger studies are required to validate the current findings aiming toward better early clinical management.

Acknowledgment

We thank the Tropical and Infectious Diseases Unit at the Royal Liverpool University Hospital for their support and help to facilitate conducting this research and overall supervision of the primary author as part of fellowship training program. We thank dr. Lamya Al Balushi from Directorate General of Health Services, Ministry of Health, Oman for her help in facilitating fieldwork.

References:

1. Lai, C.-C., et al., *Asymptomatic carrier state, acute respiratory disease, and pneumonia due to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2): Facts and myths.* Journal of Microbiology, Immunology and Infection, 2020. **53** (3): p. 404-412.

2. Yu, F., et al., *Measures for diagnosing and treating infections by a novel coronavirus responsible for a pneumonia outbreak originating in Wuhan, China.* Microbes Infect, 2020. **22** (2): p. 74-79.

3. World Health Organization. WHOCoronavirus (COVID-19) dashboard. Geneva. 2021. Available from: http://covid19.who.int. (Accesses 20th March 2021)

4. Shi, Y., et al., *COVID-19 infection: the perspectives on immune responses.* Cell Death & Differentiation, 2020. **27** (5): p. 1451-1454.

5. Bhargava, A., et al., *Predictors for Severe COVID-19 Infection.* Clin Infect Dis, 2020. **71** (8): p. 1962-1968.

6. Aziz, M., et al., *Gastrointestinal predictors of severe COVID-19: systematic review and meta-analysis.* Ann Gastroenterol, 2020.**33** (6): p. 615-630.

7. Prompetchara, E., C. Ketloy, and T. Palaga, *Immune responses in COVID-19 and potential vaccines: Lessons learned from SARS and MERS epidemic.* Asian Pac J Allergy Immunol, 2020. **38** (1): p. 1-9.

8. Chen, J., et al., *Cellular immune responses to severe acute respiratory syndrome coronavirus (SARS-CoV) infection in senescent BALB/c mice: CD4+ T cells are important in control of SARS-CoV infection.* J Virol, 2010. **84** (3): p. 1289-301.

9. Du, R.H., et al., *Predictors of mortality for patients with COVID-19 pneumonia caused by SARS-CoV-2: a prospective cohort study.*Eur Respir J, 2020. **55** (5).

10. Urra, J.M., et al., *Selective CD8 cell reduction by SARS-CoV-2 is associated with a worse prognosis and systemic inflammation in COVID-19 patients.* Clin Immunol, 2020. **217** : p. 108486.

11. Song, C.-Y., et al., *Immune dysfunction following COVID-19, especially in severe patients.* Scientific Reports, 2020.**10** (1): p. 15838.

12. Koch, S., et al., *Multiparameter flow cytometric analysis of CD4 and CD8 T cell subsets in young and old people.* Immunity & Ageing, 2008. **5** (1): p. 6.

13. Pawelec, G., *Age and immunity: What is "immunosenescence"?*Exp Gerontol, 2018. **105** : p. 4-9.

14. Li, H., B. Manwani, and S.X. Leng, *Frailty, inflammation, and immunity.* Aging Dis, 2011. **2** (6): p. 466-73.

15. De Biasi, S., et al., *Marked T cell activation, senescence, exhaustion and skewing towards TH17 in patients with COVID-19 pneumonia.* Nature Communications, 2020. **11** (1): p. 3434.

16. Kusnadi, A., et al., *Severely ill COVID-19 patients display impaired exhaustion features in SARS-CoV-2-reactive CD8⁺ T cells.*Science Immunology, 2021. **6** (55): p. eabe4782.

17. Aalto, H., et al., *Monocyte CD14 and soluble CD14 in predicting mortality of patients with severe community acquired infection.* Scandinavian Journal of Infectious Diseases, 2007.**39** (6-7): p. 596-603.

18. Payen, D., et al., *A Longitudinal Study of Immune Cells in Severe COVID-19 Patients.* Frontiers in Immunology, 2020.**11** (2759).

19. Merah-Mourah, F., et al., *Identification of Novel Human Monocyte Subsets and Evidence for Phenotypic Groups Defined by Interindividual Variations of Expression of Adhesion Molecules.*Scientific Reports, 2020. **10** (1): p. 4397.

20. Wang, Y., et al., *Clinical Course and Outcomes of 344 Intensive Care Patients with COVID-19.* Am J Respir Crit Care Med, 2020.**201** (11): p. 1430-1434.

21. Yang, J., et al., *Prevalence of comorbidities and its effects in patients infected with SARS-CoV-2: a systematic review and meta-analysis.* Int J Infect Dis, 2020. **94** : p. 91-95.

22. Ruan, Q., et al., *Clinical predictors of mortality due to COVID-19 based on an analysis of data of 150 patients from Wuhan, China.* Intensive Care Med, 2020. **46** (5): p. 846-848.

23. Wu, Z. and J.M. McGoogan, *Characteristics of and Important Lessons From the Coronavirus Disease 2019 (COVID-19) Outbreak in China: Summary of a Report of 72 314 Cases From the Chinese Center for Disease Control and Prevention.* Jama, 2020. **323** (13): p. 1239-1242.

24. Barrett, B., et al., *Inflammatory markers are poorly predictive of clinical outcomes among hospitalized patients with COVID-19.* The American journal of emergency medicine, 2020: p. S0735-6757(20)31062-7.

25. Okada, R., et al., *Phenotypic classification of human CD4+ T cell subsets and their differentiation.* Int Immunol, 2008.**20** (9): p. 1189-99.

26. Romero, P., et al., *Four Functionally Distinct Populations of Human Effector-Memory CD8⁺ T Lymphocytes.* The Journal of Immunology, 2007. **178** (7): p. 4112-4119.

27. Amyes, E., A.J. McMichael, and M.F. Callan, *Human CD4+ T cells are predominantly distributed among six phenotypically and functionally distinct subsets.* J Immunol, 2005. **175** (9): p. 5765-73.

28. Kraaijeveld, R., et al., *Co-inhibitory profile and cytotoxicity of CD57(+) PD-1(-) T cells in end-stage renal disease patients.* Clin Exp Immunol, 2018. **191** (3): p. 363-372.

29. Alshekaili, J., et al., *STAT3 regulates cytotoxicity of human CD57+ CD4+ T cells in blood and lymphoid follicles.* Scientific Reports, 2018. **8** (1): p. 3529.

30. Cunha, L.L., et al., *Remodeling of the Immune Response With Aging: Immunosenescence and Its Potential Impact on COVID-19 Immune Response.* Frontiers in Immunology, 2020. **11** (1748).

31. Giavridis, T., et al., *CAR T cell-induced cytokine release syndrome is mediated by macrophages and abated by IL-1 blockade.* Nat Med, 2018. **24** (6): p. 731-738.

32. Norelli, M., et al., *Monocyte-derived IL-1 and IL-6 are differentially required for cytokine-release syndrome and neurotoxicity due to CAR T cells.* Nat Med, 2018. **24** (6): p. 739-748.

33. Polilli, E., et al., *Comparison of Monocyte Distribution Width (MDW) and Procalcitonin for early recognition of sepsis.* PLoS One, 2020. **15** (1): p. e0227300.

34. Lippi, G. and M. Plebani, *The critical role of laboratory medicine during coronavirus disease 2019 (COVID-19) and other viral outbreaks.* Clin Chem Lab Med, 2020. **58** (7): p. 1063-1069.

35. Sanchez-Cerrillo, I., et al., *Differential Redistribution of Activated Monocyte and Dendritic Cell Subsets to the Lung Associates with Severity of COVID-19.* medRxiv, 2020: p. 2020.05.13.20100925.

36. Khartabil, T.A., et al., *A summary of the diagnostic and prognostic value of hemocytometry markers in COVID-19 patients.* Crit Rev Clin Lab Sci, 2020. **57** (6): p. 415-431.

Predictors of severe COVID-19 disease tables and figures

Table 1			
Status	**Admitted**	**Non admitted**	
Comorbid	**Counts (percentage %)**	**Counts (percentage %)**	**Pvalue**
Pregnancy	0(0)	2(4)	0.3190
Obesity	6(13)	14(26)	0.0000
Cancer	0(0)	1(2)	1.0000
Diabetes	31(65)	11(21)	0.0000
HTN	32(67)	4(8)	0.0000
HIV/ immune deficiency	2(4)	0(0)	0.2234
Heart disease	10(21)	3(6)	0.0353
Asthma	3(6)	3(6)	1.0000
Chronic lung disease	6(13)	0(0)	0.0097
Chronic liver disease	0(0)	2(4)	0.4962
Chronic haematology disease	3(6)	1(2)	0.3439
Chronic renal disease	25(52)	1(2)	0.0000
Chronic neurological disease	1(2)	0(0)	0.4752
Organ or bone marrow recipient	3(6)	0(0)	0.1038
On ACEI/ ARB	2(4)	2(4)	0.6664
On oral hypoglycemic agents/ insulin	19(40)	3(6)	0.0001
On active chemotherapy	1(2)	0(0)	0.4752
On long term steroids	4(8)	1(2)	0.1880
On biologics	1(2)	0(0)	0.4752
Symptoms			
Shortness of breath	38(79)	9	0.0000
Fever	30.00	41	0.1286
Cough	26.00	27	0.8425
Fatigue	10.00	30	0.0003
Diarrhoea	7.00	18	0.0368
Nausea/vomiting	6.00	16	0.0520
Loss of appetite	6.00	4	0.5117
Muscle/ joint pain	5.00	14	0.0454
Headache	4.00	11	0.0977
Sore throat	3.00	26	0.0000
Chills	3.00	3	1.0000
Loss of smell	2.00	25	0.0000
Seizure/ other neuro	2.00	0	0.2234
Runny Nose	1.00	23	0.0000
Rash	0.00	0	na
Epistaxis	0.00	0	na

Table 1. Comorbid conditions and symptoms of admitted (n=48) and non-admitted (n=53) patients with COVID-19. All variables reported as number (percentage). P value calculated by the Fisher's exact test, with 2×2 contingency tables.

Table 2			
Labs	**Admitted Median(IstIQ-3rdIQ)**	**Non-admitted Median(IstIQ-3rdIQ)**	**Pvalue**
Haemogloblin(g/dL)	12.15(10.35-13.38)	13.4(12.30-14.50)	0.0006
White blood cell count(10^9/L)	6.55(5.075 -8.480)	3.9(3.400-5.050)	0.0000
Platelet(10^9/L)	180(152.2245.2)	208.0(179.0-229.5)	0.3235
Neutrophil count (10^9/L)	4.9(2.950-6.475)	1.6(1.200-2.700)	0.0000
lymphocyte count (10^9/L)	1.05(0.700-1.600)	1.7(1.300-2.050)	0.0003
Neutrophil/ lymphocyte ratio	3.775(2.075-6.125)	1.05(0.605-1.775)	0.0000
CRP (mg/L)	114(53.8 -176.4)	4(4-23.2)	0.0000
LDH	416.5(321.5-528.0)	214(187.0-246.0)	0.0000
Ferritin	760(312-1645)	196(54-346)	0.0000
Urea (mmol/L)	10.3(4.15-40.30)	3.9(3.200-4.600)	0.0000
Creatinine (mmol/L)	151(69.5-484.0)	66.0(53.0-75.0)	0.0000
eGFR mL/min/1.73 m^2	43(9.00-90.00)	90.00(90.00-90.00)	0.0000
ALT (U/L)	30.5(21.0-63.0)	27.00(18.75- 47.25)	0.4888
AST (U/L)	39.0(27.5-100.5)	27.00(21.50-35.00)	0.0283
ALP (U/L)	79(62.5-132.0)	74.00(59.50-90.50)	0.1360
GGT (U/L)	47.5(34.25 -195.50)	36.00(21.50-49.00)	0.0644
Bilirubin (umol/L)	7.00(5.00-10.75)	8.00(5.750-10.000)	0.3394
Albumin (g/L)	38(35.00-40.00)	44.50(42.00-46.00)	0.0000
PT (second)	11.1(10.90-12.18)	10.5(10.30-10.88)	0.0000
APTT (second)	35.15(32.23-38.00)	28.65(26.80-31.48)	0.0000
Fibrinogen (g/L)	5.585(3.752-6.718)	3.340(2.803-4.240)	0.0000
D-dimer mg/L FEU	1.07(0.780-4.130)	0.31(0.2325-0.5975)	0.0000
DIC score	2(0-3)	0(0-0)	0.0000

Table 2. Laboratory findings of the admitted (n=48) and non-admitted (n=53) patients with COVID-19. All variables reported as median (1st and 3rd quartile). P value calculated by the non-parametric U Mann-Withney test. Serum aspartate aminotransferase (AST), serum alanine aminotransferase (ALT), C-reactive protein (CRP), lactate dehydrogenase (LDH), Alkaline phosphatase (ALP), Gamma-glutamyl transferase (GGT), prothrombin time (PT), or activated partial thromboplastin time (aPTT), disseminated intravascular coagulation score (DIC score).

Table 3			
Immune markers	**Admitted Median(IstIQ-3rdIQ)**	**Non-admitted Median(IstIQ-3rdIQ)**	**Pvalue**
IL-6	33(8.36-86.28)	3.71(1.58-12.580)	0.0000
CD3+	62.03(53.18-71.11)	70.85(64.30 -73.63)	0.0100
CD4+	56.99(50.34-67.47)	61.47(52.46-66.14)	0.3166
CD8+	34.4(25.12-42.83)	31.88(26.09-38.28)	0.3166
CD19+	14.22(7.275-21.545)	11.23(8.01-13.69)	0.2922
CD56+	18.27(9.90 -26.58)	15.33(11.17-20.67)	0.2986
TEM CD4+	13.3(6.82-22.42)	10.33(8.21-14.27)	0.1432
Naïve CD4+	25.4(14.99-36.88)	32.75(23.51-39.50)	0.0343
TCM CD4+	50.91(43.31-42.83)	53.02(46.72-58.33)	0.7299
TEMRA CD4+	0.37(0.0551.510)	0.17(0.050-0.800)	0.3368
CD27-CD28-Naïve CD4+	0.03(0.0000-0.2050)	0.02(0.00-0.05)	0.4492
CD27-CD28+Naïve CD4+	0.81(0.195-1.725)	0.34(0.18-0.65)	0.0548
CD27+CD28-Naïve CD4+	0.19(0.0650 -0.4500)	0.11(0.04-0.2300)	0.0397
CD27+CD28+Naïve CD4+	99(97.15-99.67)	99.48(98.90- 99.71)	0.0213
CD27-CD28-TCM CD4+	0.54(0.115-1.580)	0.29(0.05- 1.07)	0.4597
CD27-CD28+TCM CD4+	5.68(4.885-7.635)	6.57(4.22-7.97)	0.4769
CD27+CD28-TCM CD4+	0.15(0.050-0.400)	0.09(0.04-0.19)	0.1800
CD27+CD28+TCM CD4+	93.24(89.9494.59)	92.55(91.38- 94.62)	0.9257
CD27-CD28-TEM CD4+	22.3(4.98-42.01)	8.46(2.44-29.36)	0.0698
CD27-CD28+TEM CD4+	27.34(20.20-35.31)	31.99(22.05-37.79)	0.1589
CD27+CD28-TEM CD4+	0.79(0.285-1.745)	0.76(0.35-1.42)	0.8819
CD27+CD28+TEM CD4+	47.37(25.23-58.87)	52.47(39.59-61.43)	0.0737
CD27-CD28-TEMRA CD4+	51.92(0.0079.48)	50.00(11.99 -77.08)	0.8673
CD27-CD28+ TEMRA CD4+	12(2.35-23.09)	18.00(6.07-27.75)	0.3042
CD27+CD28- TEMRA CD4+	3.685(0.000-9.557)	3.23(0.00-6.02)	0.3909
CD27+CD28+ TEMRA CD4+	10.265(1.808-39.200)	19.23(3.53-51.09)	0.2330
PD-1-CD57+CD4+	1.46(0.385- 3.590)	0.89(0.50-3.64)	0.9285
PD-1+CD57-CD4+	17.59(14.16-23.48)	13.63(10.24-18.20)	0.0007
PD-1+CD57+CD4+	5.48(1.660-12.885)	2.97(1.56-5.98)	0.0396
CD45RA-PD1+CD4+	13.48(9.295-20.300)	12.57(8.19-17.09)	0.4413
Naïve CD8+	14.31(4.485-36.240)	27.82(14.84-37.89)	0.0099
TCM CD8+	8.64(4.715-13.830)	10.5(7.58-16.86)	0.0523
TEM CD8+	30.23(18.20-40.65)	29.26(22.32-37.58)	0.9394
TEMRA CD8+	31.85 (16.54-45.24)	23.46(15.30 -31.33)	0.0731
CD27-CD28-Naïve CD8+	00(00-0.855)	0.07(0.00-0.33)	0.3339
CD27-CD28+Naïve CD8+	0.270 (0.065-.020)	0.19(0.09-0.41)	0.3816
CD27+CD28-Naïve CD8+	3.92(2.440-7.055)	2.67(1.22-5.79)	0.1244
CD27+CD28+Naïve CD8+	94.93(87.39-97.33)	96.2(90.48-98.47)	0.0913
CD27-CD28-TCM CD8+	1.67(0.720 -5.570)	0.74(0.40-2.14)	0.0262
CD27-CD28+TCM CD8+	2.06(1.140-4.320)	1.21(0.82-2.56)	0.0486
CD27+CD28-TCM CD8+	5.63(3.450-8.765)	5.33(3.29-7.57)	0.7533
CD27+CD28+TCM CD8+	88.88(83.05-93.55)	91.69(87.16-94.55)	0.0688
CD27-CD28-TEM CD8+	47.25(24.14-57.68)	25.76(15.45-40.84)	0.0017
CD27-CD28+TEM CD8+	6.41(3.635-10.930)	6.26(4.81- 9.65)	0.6761
CD27+CD28-TEM CD8+	12.95(7.86-20.89)	15.99(12.64- 20.75)	0.1252
CD27+CD28+TEM CD8+	28.71(21.38-45.66)	43.95(32.92-59.75)	0.0007
CD27-CD28-TEMRA CD8+	76.65(57.97-87.09)	71.34(61.51-79.33)	0.1413
CD27-CD28+ TEMRA CD8+	1.31(0.550-3.255)	0.91(0.41-1.65)	0.1460
CD27+CD28- TEMRA CD8+	16.33(10.29-27.73)	21.07(15.00-28.39)	0.0286
CD27+CD28+ TEMRA CD8+	4.13(1.590-9.585)	5.10(3.10-10.24)	0.1906
PD-1-CD57+CD8+	14.91(9.49-26.96)	13.10(6.22-23.24)	0.1499
PD-1+CD57-CD8+	24.34(20.34-33.40)	29.70(23.13-34.56)	0.1010
PD-1+CD57+CD8+	16.86(11.35-27.96)	11.98(8.96-15.41)	0.0078
CD3+CD4-CD8-	4.08(3.005-6.195)	4.55(3.69-6.44)	0.2362
CD16-CD56+	3.01(2.285-4.370)	3.07(2.28-5.82)	0.5297
CD16+CD56+	95.3(92.88-96.60)	95.06(92.31-96.03)	0.4171
CD14+	3.91(2.195-6.310	7.43(6.09-10.10)	0.0000
LCD14+	0.59(0.305 -1.020)	0.64(0.44-1.03)	0.2038
LCD16-CD14+	72.7(59.38 -87.69)	84.71(80.46-88.61)	0.0008
LCD16+CD14+	27.3(12.31-40.62)	15.29(11.39- 9.54)	0.0009
SCD14+	2.46(1.210-4.100)	4.58(3.34-5.53)	0.0000
sCD16-CD14+	81.52(71.72-88.07)	83.16(73.99-89.12)	0.9422
sCD16+CD14+	18.48(11.93-27.70)	16.84(10.88-26.01)	0.9257

Table 3. Immunological findings of the admitted (n=48) and non-admitted (n=53) patients with COVID-19. All variables reported as median (1st and 3rd quartile). P value calculated by the non-parametric U Mann-Withney test.

Table 4

	Uni-variable analysis	Multi-variable analysis	
Variable	**P value**	**OR**	**P value**
Age	0.022	0.924	0.089
Gender (%Male)	0.471		
BMI	0.516		
admission status	0.010	1.208	0.921
Diabetes	0.020	0.268	0.340
HTN	0.024		
HIV/ immune deficiency	0.152		
Heart disease	0.621		
Asthma	0.711		
Chronic lung disease	0.711		
Chronic renal disease	0.524		
White blood cell count(10^9/L)	0.211		
Neutrophil count (10^9/L)	0.104		
lymphocyte count (10^9/L)	0.053		
Neutrophil/ lymphocyte ratio	0.181		
CRP (mg/L)	0.044		
LDH	0.014		
Ferritin	0.003		
Fibrinnogen	0.914		
D-dimer	0.039		
IL-6	0.001	1.030	0.032
CD3+	0.143		
CD4+	0.303		
CD8+	0.556		
TEM CD4+	0.869		
Naïve CD4+	0.281		
TCM CD4+	0.101		
TEMRA CD4+	0.211		
CD27+CD28+Naïve CD4+	0.005	0.476	0.030
CD27+CD28+TCMD4+	0.460		
CD27-CD28+TEM CD4+	0.862		
CD27+CD28+TEM CD4+	0.334		
Naïve CD8+	0.593		
TCM CD8+	0.100		
TEM CD8+	0.377		
TEMRA CD8+	0.489		
CD27+CD28+Naïve CD8+	0.212		
CD27+CD28+TCM CD8+	0.168		
CD27-CD28-TEM CD8+	0.913		
CD27-CD28+TEM CD8+	0.303		
CD27+CD28-TEM CD8+	0.133		
CD27+CD28+TEM CD8+	0.524		
CD27-CD28-TEMRA CD8+	0.328		
CD27-CD28+TEMRA CD8+	0.488		
CD27+CD28-TEMRA CD8+	0.476		
CD27+CD28+TEMRA CD8+	0.345		
PD-1-CD57+ CD4+	0.368		
PD-1+CD57-CD4+	0.572		
PD-1+CD57+ CD4+	0.748		
CD45RA-PD-1+ CD4+	0.035	1.139	0.055
PD-1-CD57+ CD8+	0.332		
PD-1+CD57- CD8+	0.531		
PD-1+CD57+ CD8+	0.076	0.893	0.110
CD14	0.022	0.862	0.437
LCD14	0.614		
LCD16nCD14p	0.000		
LCD16pCD14p	0.000	1.117	0.010
SCD14	0.021		
sCD16nCD14p	0.521		
sCD16pCD14p	0.631		

Table 4. Univariable and Multivariable analysis of the selected parameters. Odd ratio (OR).

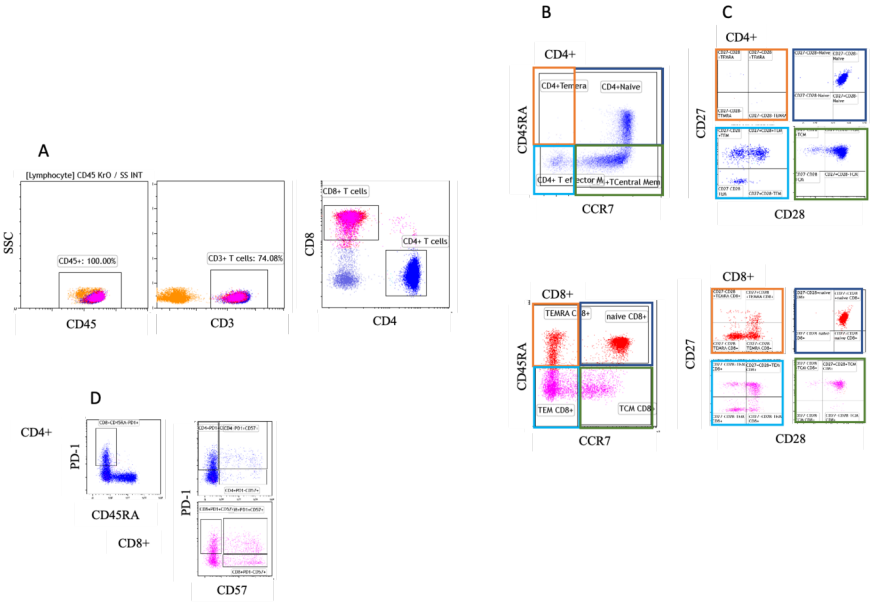

Figure 1. Distribution of CD4 and CD8 T subsets in the blood.

(A) Representative flow cytometric analysis of the gating strategy, leukocytes (CD45+), T cell (CD3+) and CD4 and CD8 T cells. (B) Based on CD45RA and CCR7 expression, CD4 and CD8 have 4 main subsets; naïve (CD45RA+CCR7+), central memory (TCM, CD45RA-CCR7+), effector memory (TEM, CD45RA-CCR7-), revertant effector memory TEMRA (CD45RA+CCR7-). CD4+ subset in the upper panel and CD8+subsets in the lower panel. (C) The four main subsets (Naïve (dark blue), TCM (green), TEM (light blue) and TEMRA (orange) are further divided into a different subset based on surface expression of CD27 and CD28. (D) CD4 is separated into T follicular helper cells (PD-1+CD45RA-). CD4 and CD8 are divided into cytotoxic (PD-1+CD57+) and senescence cells (PD-1-CD57+). CD4+ subset in the upper panel and CD8+subsets in the lower panel.

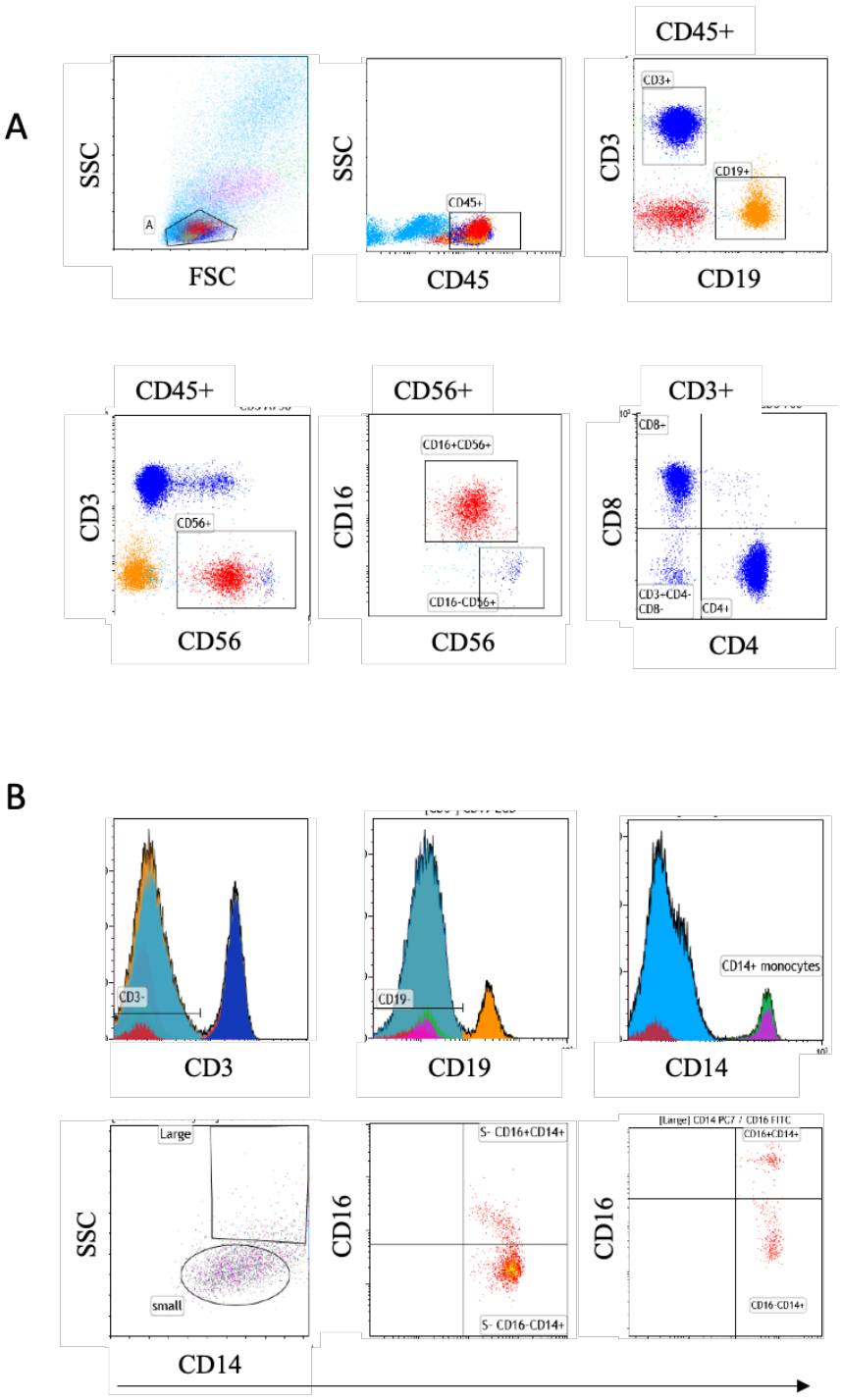

Figure 2. Distribution of lymphocytes and monocytes. (A) Representative flow cytometric analysis of the gating strategy, leukocytes (CD45+), T cell (CD3+), B cells (CD19), NK (CD56), and CD4 and CD8 T cells. (B) Gating on CD3- followed by gating on CD19- and then CD14+ (upper raw). Based on size of CD14+ monocytes into small and large followed by expression of CD16 (lower raw).

Hosted file

`April 17th cover letter.docx` available at https://authorea.com/users/729123/articles/709756-immunological-predictors-of-disease-severity-in-patients-with-covid-19-infection

Hosted file

`April 17th tables and figures.docx` available at https://authorea.com/users/729123/articles/709756-immunological-predictors-of-disease-severity-in-patients-with-covid-19-infection