Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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Immunological predictors of disease severity in patients with COVID-19

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ABSTRACT

Background: Identifying the immune cells involved in coronavirus disease 2019 (COVID-19) disease progression and the predictors of poor outcomes is important to manage patients adequately.

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

Results: Hospitalized patients were older (58 years vs 36 years, $P < 0.001$) and had more comorbid conditions such as diabetes (65% vs 21% $P < 0.001$). Hospitalized patients had significantly higher inflammatory markers ($P < 0.001$): C-reactive protein (114 vs 4 mg/l), interleukin 6 (IL-6) (33 vs 3.71 pg/ml), lactate dehydrogenase (417 vs 214 U/l), ferritin (760 vs 196 ng/ml), fibrinogen (6 vs 3 g/l), D-dimer (1.0 vs 0.3 μg/ml), disseminated intravascular coagulopathy score (2 vs 0), and neutrophil/lymphocyte ratio (4 vs 1.1) ($P < 0.001$). On multivariate regression analysis, statistically significant independent early predictors of intensive care unit admission or death were higher levels of IL-6 (odds ratio 1.03, $P = 0.03$), frequency of large inflammatory monocytes (CD14+CD16+) (odds ratio 1.117, $P = 0.010$), and frequency of circulating naïve CD4+ T cells (CD27+CD28+CD45RA+CCR7+) (odds ratio 0.476, $P = 0.03$).

Conclusion: IL-6, the frequency of large inflammatory monocytes, and the frequency of 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.

1. Introduction

A cluster of atypical viral pneumonia cases was identified in Wuhan, China, in December 2019. A novel coronavirus was identified as the cause, later named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (Lai et al., 2020; Yu et al., 2020). The World Health Organization (WHO) declared it a pandemic on March 11, 2020, and by March 20, 2021, the total number of confirmed cases had exceeded 121 million worldwide, with over 2.6 million deaths (Shi et al., 2020). Some people infected with SARS-CoV-2 develop severe coronavirus disease 2019 (COVID-19) while others remain asymptomatic or have a milder illness course

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(Shi et al., 2020). Identifying predictors of poor outcomes is increasingly gaining importance to help to prioritize resources for high-risk patients and minimize death. Older age and certain comorbid conditions like chronic renal, lung, and heart diseases are established predictors of a 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 also predictors of a worse prognosis (Bhargava et al., 2020; Aziz et al., 2020; Shi et al., 2020; Prompetchara et al., 2020).

In these patients, immune cells, namely lymphocytes, have been heavily implicated in controlling disease progression and clinical outcomes. Some studies have demonstrated that higher leukocyte counts, specifically neutrophils (Shi et al., 2020; Chen et al., 2010; Prompetchara et al., 2020), and T cell lymphopenia (CD3+, CD8+ (Du et al., 2020; Urra et al., 2020), and CD4 (Song et al., 2020)) are associated with increased mortality in patients admitted with COVID-19 pneumonia. Moreover, it has been shown that older patients have lower counts and frequencies of naïve (CD45RA+CCR7+CD27+CD28+) CD4+ T cells contributing to the poor response of T cells (Koch et al., 2008). These cells are required for the effective handling of new infections or vaccines (Pawelec 2018; Li et al., 2011). It has also been shown that hospitalized COVID-19 patients have reduced (CD45RA+CCR7+CD27+CD28+) CD4+ naïve subsets of T cells compared to healthy uninfected controls (De Biasi et al., 2020). Furthermore, hospitalized patients with severe manifestations have a lower frequency of exhausted non-cytotoxic T cells (PD-1+CD57−CD8+) (Kusnadi et al., 2021).

Monocytes are other immune cells that are also vital for a normal and dysregulated immune response. Monocytopenia was found to be a predictor of worse outcomes in patients with severe community infections and sepsis (Aalto et al., 2007). Moreover, there is a reduction in the classic monocytes (CD14+CD16−) in severe SARS-CoV-2 infection and an increase in the inflammatory subsets (CD14+CD16+) (Payen et al., 2020). 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 (Merah-Mourah et al., 2020).

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

2. Methods

2.1. Study design and patients

This was a prospective observational cohort study conducted from July 20, 2020 to August 27, 2020. A total of 101 COVID-19 cases confirmed by SARS-CoV-2 real-time PCR (RT-PCR) from nasopharyngeal swabs were enrolled: 53 non-hospitalized cases and 48 hospitalized cases. Inclusion criteria were patient age ≥13 years, both sexes, confirmed mild SARS-CoV-2 infection in the non-hospitalized group, and confirmed moderate infection in patients hospitalized at the Royal Hospital. 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 that 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 dedicated 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. Telephone calls were conducted to obtain patient 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. Patient demographics and clinical characteristics were obtained from non-hospitalized patients directly or for hospitalized patients through the electronic hospital records using a unified data collection form. Informed consent was obtained from all enrolled patients. The study was approved by the Central Research Committee at the Ministry of Health in Oman (MoH/CSR/20/23605).

2.2. Measurement of inflammatory markers and lymphocyte subsets

Blood was collected at a median 6 days (interquartile range (IQR) 2–8 days) from the onset of symptoms for the hospitalized patients and 7.5 days (IQR 6.75–8.25 days) for the non-hospitalized patients. . Blood was sent for complete blood count, renal function tests, liver function tests, lactate dehydrogenase (LDH), ferritin, D-dimer, coagulation profile, IL-6, and lymphocyte subset analysis. The IL-6 concentration was measured on serum samples using the fully automated Elecsys IL-6 immunoassay (electrochemiluminescence immunoassay) on a Cobas e601 immunoassay analyzer (Roche Diagnostics, Switzerland) following the manufacturer's protocol. The IL-6 cut-off used was 7.0 pg/ml.

The assessment of the different basic lymphocyte and detailed T cell subsets was performed using flow cytometry. The DURAClone IM T cell subsets tube (Beckman Coulter) that includes CD45RA (clone, 2H4), CD197 (CCR7) (clone, G043H7), CD28 (clone, CD28.2), CD279 (PD-1) (clone, PD-1.3.5), CD27 (clone, 1A4.CD27), CD4 (clone, 13B8.2), CD8 (clone, B9.11), CD3 (clone, UCHT-1), CD57 (clone, NC1), and CD45 (clone, J33) was used to assess the different T cell subsets. The 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), and CD45 (J33) was used for basic lymphocyte staining. Gating strategies are presented in Figures 1 and 2. A total of 100 000 events were collected.

Briefly, 100 μl of blood was added to the tube containing the desired cocktail of antibodies and incubated for 20 min at room temperature. Next, 100 μl of lysing solution OptiLyse B or VersaLyse was added according to the manufacturer's recommendation. This was followed by a wash step. The acquisition of samples was done using Navios flow cytometry (Beckman Coulter) and the analysis was performed using Kaluza version 2.1 (Beckman Coulter).

2.3. Statistical analysis

Results were expressed as the median and interquartile range (IQR) and the frequency and percentage (%) for continuous and categorical variables, respectively. The assessment of differences between inpatients and outpatients was performed using the Chi-square test or Fisher's exact test for categorical variables, and the Mann–Whitney *U*-test for continuous variables.

Selected variables from the demographic characteristics, clinical presentation, inflammatory markers, and all significant immune subsets were subjected to univariable logistic regression analysis with the composite outcome of ICU admission and mortality in the first 30 days. Variables significant in the univariable analysis ($P <$

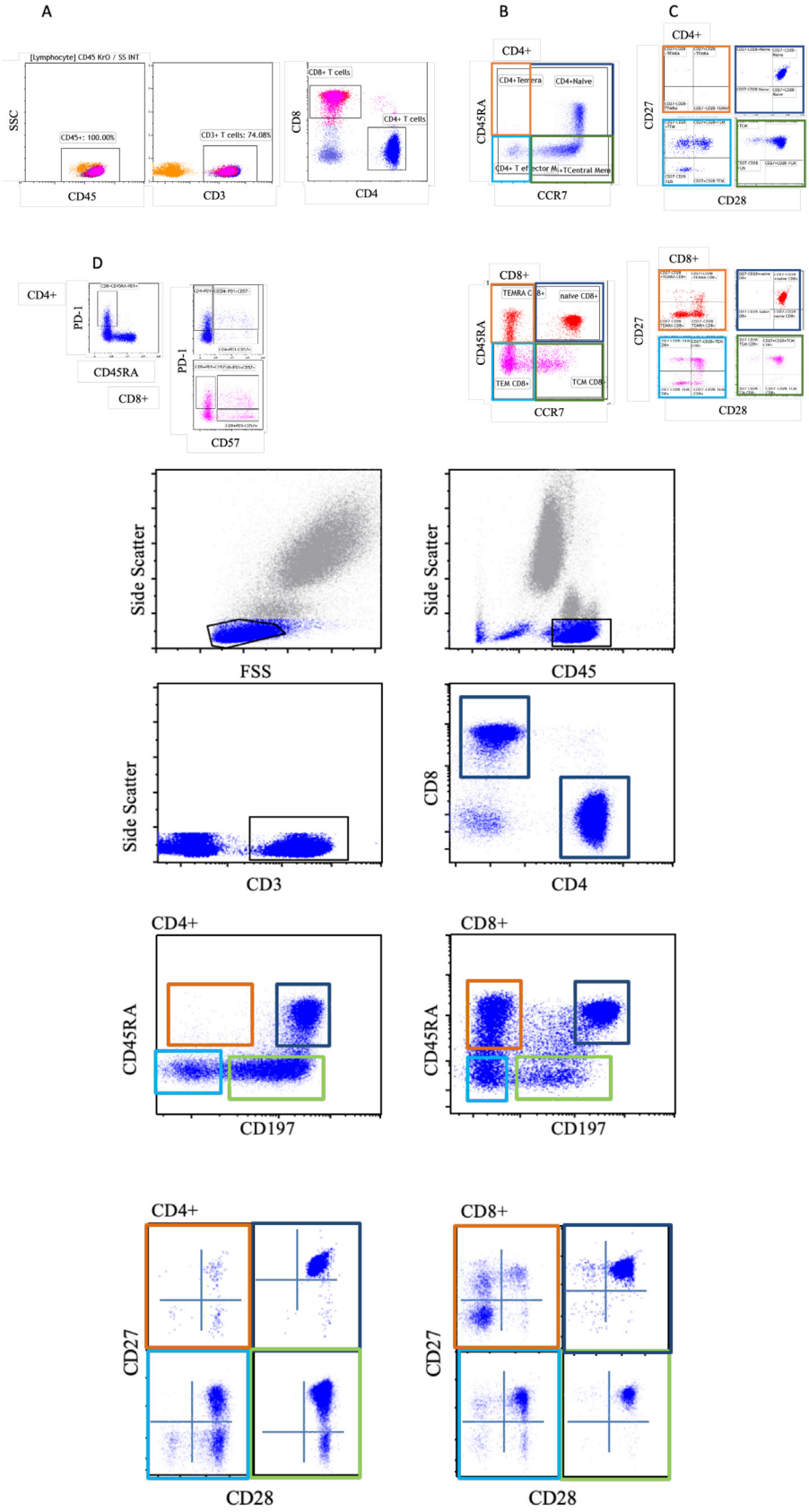

Figure 1. Distribution of CD4 and CD8 T cell subsets in the blood. (A) Representative flow cytometry analysis of the gating strategy, leukocytes (CD45+), T cell (CD3+), and CD4 and CD8 T cells. (B) Based on CD45RA and CCR7 expression, CD4 and CD8 have four main subsets: naïve (CD45RA+CCR7+), central memory (TCM, CD45RA−CCR7+), effector memory (TEM, CD45RA−CCR7−), and 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 left panel and CD8+ subsets in the right panel.

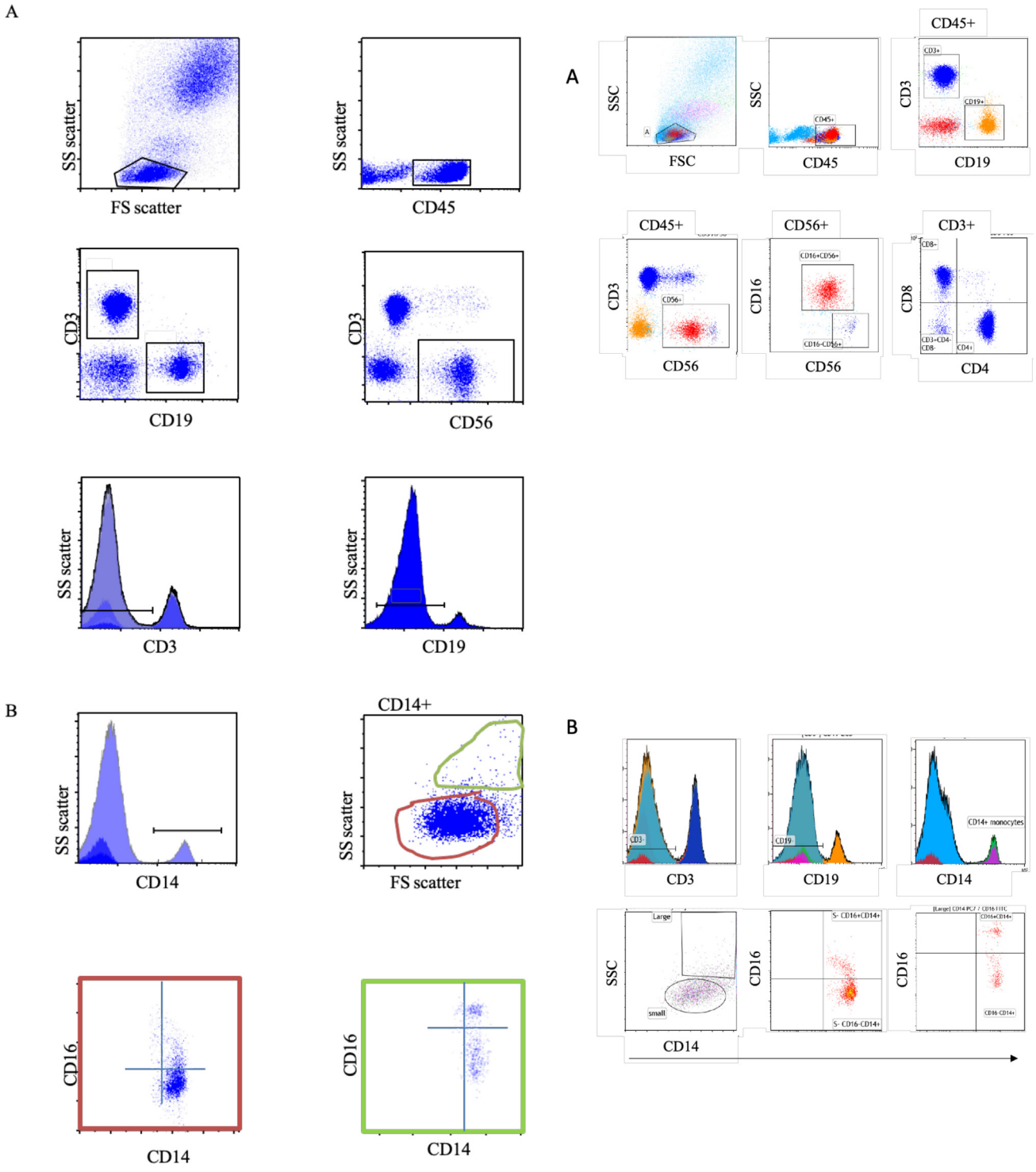

Figure 2. Distribution of lymphocytes and monocytes. (A) Representative flow cytometry analysis of the gating strategy, leukocytes (CD45+), T cell (CD3+), B cell (CD19), NK cell (CD56), and CD4 and CD8 T cells. (B) Gating on CD3− followed by gating on CD19− and then CD14+ (upper two rows). Based on the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

0.05) were assessed using multivariable logistic regression analysis to determine the independent predictors of the composite outcome.

An alpha threshold of 0.05 was used for statistical significance. The statistical analysis was performed using RStudio (RStudio Team (2016), RStudio (version 1.1.456): Integrated Development for R; RStudio, Inc., Boston, MA, USA).

3. Results

3.1. Demographic data, clinical characteristics, and main laboratory findings

During the period between July 2020 and August 2020, a total of 53 non-hospitalized patients with mild SARS-CoV-2 infection

Table 1
Comorbid conditions and symptoms of hospitalized ($n = 48$) and non- hospitalized ($n = 53$) patients with COVID-19. All variables are reported as the number and percentage (%). The *P*-values were calculated using Fisher's exact test, with 2 × 2 contingency tables

	Status		*P*-value
	Hospitalized*n* (%)	Non-hospitalized*n* (%)	
Comorbid conditions			
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 hematological 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 (17)	0.0000
Fever	30.(62.5%)	41 (77.4)	0.1286
Cough	26.00 (54.17)	27 (50.9)	0.8425
Fatigue	10.00 (20.83)	30 (56.6)	0.0003
Diarrhea	7.00 (14.58)	18 (34)	0.0368
Nausea/vomiting	6.00 (12.5)	16 (30.2)	0.0520
Loss of appetite	6.00 (12.5)	4 (7.5)	0.5117
Muscle/joint pain	5.00 (10.42)	14 (26.4)	0.0454
Headache	4.00 (8.33)	11 (20.8)	0.0977
Sore throat	3.00 (6.25)	26 (49.1)	0.0000
Chills	3.00 (6.25)	3 (5.7)	1.0000
Loss of smell	2.00 (4.17)	25 (47.2)	0.0000
Neurological deficits or seizure	2.00 (4.17)	0 (0)	0.2234
Runny nose	1.00 (2.8)	23 (43.4)	0.0000
Rash	0.00 (0)	0 (0)	NA
Epistaxis	0.00	0	NA

ACEI, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker; HTN, hypertension; NA, not applicable.

and 48 hospitalized patients with moderate SARS-CoV-2 infection were recruited. Demographic and 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 ($P < 0.001$). However, there was no difference in sex distribution (male: 54% of hospitalized vs 43% of non-hospitalized, $P = 0.32$). Comorbid conditions were more frequent in the admitted group, such as diabetes (65% vs 21%, $P < 0.001$). 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 (Table 1, **Supplementary Material** Appendix 1).

The 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 μg/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, $P < 0.001$) (Table 2, **Supplementary Material** Appendix 1).

3.2. Immunological features

3.2.1. CD3 lymphopenia in the hospitalized group

Despite having higher total white blood cell and neutrophil counts (Table 2), hospitalized patients had a lower lymphocyte count and a lower percentage of the CD3+ T cell subset ($P = 0.001$). At the same time, the ratio of CD4+ T cells and CD8+ T cells was normal (Table 3), and there was no difference between the two groups when comparing the percentage of CD19+ B cells and CD16+56+ natural killer (NK) cells ($P = 0.29$ and $P = 0.42$, respectively) (Table 3, **Supplementary Material** Appendix 1).

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

The assessment of the CD4+ T cell maturation and differentiation stages 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% (IQR 14.99–36.88%)) 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% (IQR 98.90–99.71%) vs 99.00% (IQR 97.15–99.67%) in the non-hospitalized group and 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%) compared to 13.63% (IQR 10.24–18.20%) ($P = 0.001$) and exhausted cytotoxic (PD-1+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%) in the non-hospitalized patients ($P = 0.039$) (Table 3, **Supplementary Material** Appendix 1).

Table 2
Laboratory findings of the hospitalized ($n = 48$) and non-hospitalized ($n = 53$) patients with COVID-19. All variables are reported as the median (interquartile range). The *P*-value was calculated by non-parametric Mann–Whitney *U*-test

Laboratory tests	HospitalizedMedian (IQR)	Non-hospitalizedMedian (IQR)	*P*-value
Hemoglobin (g/dl)	12.15 (10.35–13.38)	13.4 (12.30–14.50)	0.0006
White blood cell count ($\times 10^9$/l)	6.55 (5.07–8.48)	3.9 (3.40–5.05)	0.0000
Platelet count ($\times 10^9$/l)	180 (152.2–245.2)	208.0 (179.0–229.5)	0.3235
Neutrophil count ($\times 10^9$/l)	4.9 (2.95–6.47)	1.6 (1.20–2.70)	0.0000
lymphocyte count ($\times 10^9$/l)	1.05 (0.70–1.60)	1.7 (1.30–2.05)	0.0003
Neutrophil/lymphocyte ratio	3.775 (2.07–6.12)	1.05 (0.60–1.77)	0.0000
CRP (mg/l)	114 (53.8–176.4)	4 (4–23.2)	0.0000
LDH (U/l)	416.5 (321.5–528.0)	214 (187.0–246.0)	0.0000
Ferritin (ng/ml)	760 (312–1645)	196 (54–346)	0.0000
Urea (mmol/l)	10.3 (4.15–40.30)	3.9 (3.20–4.60)	0.0000
Creatinine (mmol/l)	151 (69.5–484.0)	66.0 (53.0–75.0)	0.0000
eGFR (ml/min/1.73m^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 (μmol/l)	7.00 (5.00–10.75)	8.00 (5.75–10.00)	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 (3.75–6.71)	3.340 (2.80–4.24)	0.0000
D-dimer (mg/l FEU)	1.07 (0.78–4.13)	0.31 (0.232–0.597)	0.0000
DIC score	2 (0–3)	0 (0–0)	0.0000

ALP, alkaline phosphatase; ALT, serum alanine aminotransferase; aPTT, activated partial thromboplastin time; AST, serum aspartate aminotransferase; CRP, C-reactive protein; DIC, disseminated intravascular coagulation; eGFR, estimated glomerular filtration rate; FEU, fibrinogen-equivalent units; GGT, gamma-glutamyl transferase; IQR, interquartile range; LDH, lactate dehydrogenase; PT, prothrombin time.

3.2.3. 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% (IQR 14.84–37.89%) compared to14.31% (IQR 4.485–36.24%) in the hospitalized group ($P = 0.010$). In addition, the non-hospitalized group had a higher frequency of cells with lower cytotoxic characteristics. Examples include effector memory (TEM) CD27+CD28+CD8+ T cells (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, **Supplementary Material** Appendix 1).

In contrast, the 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, the percentage of cytotoxic exhausted (CD57+PD-1+) CD8+ T cells was 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, **Supplementary Material** Appendix 1). The hospitalized group had a higher frequency of cells with cytotoxic characteristics.

3.2.4. Large inflammatory (CD14+CD16+) monocytes in the hospitalized group

Hospitalized patients exhibited a lower percentage of CD14+ monocytes than non-hospitalized patients, with a median of 3.91% (2.20–6.31%) vs 7.43% (IQR 6.09–10.10%), respectively ($P < 0.001$). As a 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 group 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 the non-hospitalized group with 15.29% (IQR 11.39–9.54%) ($P < 0.001$) (Table 3, **Supplementary Material** Appendix 1).

3.2.5. 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 pg/ml) compared to the non-hospitalized group with 3.71 pg/ml (IQR 1.58–12.58 pg/ml) ($P < 0.001$) (Table 3, **Supplementary Material** Appendix 1).

3.2.6. ICU admission and death

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

3.2.7. 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, **Supplementary Material** Appendix 1). Significant univariable factors were then subjected to multivariable regression analysis. It was found that the increase in IL-6 level (pg/ml) increased the composite endpoint odds by 1.03 ($P = 0.03$). Similarly, an increase in the percentage of the large inflammatory monocytes (CD16+CD14+) subset was found to be associated with an increase in the composite endpoint odds by 1.117 ($P = 0.01$). On the other hand, an increase in the frequency of the naïve CD4+ (CD45RA+CCR7+CD27+CD28+) decreased the odds of the composite endpoint by 0.476 ($P = 0.03$) (Table 4, **Supplementary Material** Appendix 1).

4. Discussion

Earlier studies have shown that in hospitalized patients, inflammatory biomarkers such as CRP, ferritin, LDH, D-dimer, and

Table 3
Immunological findings of the hospitalized ($n = 48$) and non-hospitalized ($n = 53$) patients with COVID-19. All variables are reported as the median (interquartile range). The *P*-value was calculated by non-parametric Mann–Whitney *U*-test

Immune markers	HospitalizedMedian (IQR)	Non-hospitalizedMedian (IQR)	*P*-value
IL-6	33 (8.36–86.28)	3.71 (1.58–12.58)	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 (7.27–21.54)	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 (0.05–1.51)	0.17 (0.05–0.80)	0.3368
CD27−CD28−Naïve CD4+	0.03 (0.000.20)	0.02 (0.00–0.05)	0.4492
CD27−CD28+Naïve CD4+	0.81 (0.19–1.72)	0.34 (0.18–0.65)	0.0548
CD27+CD28−Naïve CD4+	0.19 (0.06–0.45)	0.11 (0.04–0.23)	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 (0.11–1.58)	0.29 (0.05–1.07)	0.4597
CD27−CD28+TCM CD4+	5.68 (4.88–7.63)	6.57 (4.22–7.97)	0.4769
CD27+CD28−TCM CD4+	0.15 (0.05–0.40)	0.09 (0.04–0.19)	0.1800
CD27+CD28+TCM CD4+	93.24 (89.94–94.59)	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 (0.28–1.74)	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 (0.00–79.48)	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 (0.00–9.55)	3.23 (0.00–6.02)	0.3909
CD27+CD28+ TEMRA CD4+	10.265 (1.80–39.20)	19.23 (3.53–51.09)	0.2330
PD-1−CD57+CD4+	1.46 (0.38–3.59)	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 (1.66–12.88)	2.97 (1.56–5.98)	0.0396
CD45RA−PD-1+CD4+	13.48 (9.29–20.30)	12.57 (8.19–17.09)	0.4413
Naïve CD8+	14.31 (4.48–36.24)	27.82 (14.84–37.89)	0.0099
TCM CD8+	8.64 (4.71–13.83)	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 (00–0.85)	0.07 (0.00–0.33)	0.3339
CD27−CD28+Naïve CD8+	0.270 (0.06–1.020)	0.19 (0.09–0.41)	0.3816
CD27+CD28−Naïve CD8+	3.92 (2.44–7.05)	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 (0.72–5.57)	0.74 (0.40–2.14)	0.0262
CD27−CD28+TCM CD8+	2.06 (1.14–4.32)	1.21 (0.82–2.56)	0.0486
CD27+CD28−TCM CD8+	5.63 (3.45–8.76)	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 (3.63–10.93)	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 (0.55–3.25)	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 (1.59–9.58)	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
CD45RA−PD-1+CD8+	21.48 (13.53–33.84)	13.88 (11.78–19.79)	0.0023
CD3+CD4−CD8−	4.08 (3.00–6.19)	4.55 (3.69–6.44)	0.2362
CD16−CD56+	3.01 (2.28–4.37)	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 (2.19–6.31)	7.43 (6.09–10.10)	0.0000
LCD14+	0.59 (0.30–1.02)	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 (1.21–4.10)	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

IL-6, interleukin 6; IQR, interquartile range.

IL-6 can be used to predict clinical outcomes in COVID-19 patients (Bhargava et al., 2020; Aziz et al., 2020; Shi et al., 2020; Prompetchara et al., 2020). The immune system plays a significant role in the clinical manifestations and progression of the disease, including the inflammatory markers mentioned above. Therefore, the focus on immunological predictors that can be used early in the disease course to enable the relocation of resources towards those at risk of getting the severe disease should be prioritized. In this study, it was observed that, in addition to elevated levels of IL-6, the higher percentage of large inflammatory CD14+CD16+ monocytes and lower percentage of naïve CD27+CD28+CD4+ T cells were independent early immunological

Table 4
Univariable and multivariable analysis of the selected parameters

Variable	Univariable analysis	Multivariable analysis	
	P-value	OR	*P*-value
Age	0.022	0.924	0.089
Sex (% 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 ($\times 10^9$/l)	0.211		
Neutrophil count ($\times 10^9$/l)	0.104		
lymphocyte count ($\times 10^9$/l)	0.053		
Neutrophil/lymphocyte ratio	0.181		
CRP (mg/l)	0.044		
LDH (U/l)	0.014		
Ferritin (ng/ml)	0.003		
Fibrinogen	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
Large CD14	0.614		
Large CD16nCD14p	0.000		
Large CD16pCD14p	0.000	1.117	0.010
Small CD14	0.021		
Small CD16nCD14p	0.521		
Small CD16pCD14p	0.631		

BMI, body mass index; CRP, C-reactive protein; HTN, hypertension; IL-6, interleukin 6; LDH, lactate dehydrogenase; OR, odds ratio.

prognostic predictors of worse outcomes in patients with COVID-19.

Similar to other existing data, admitted patients in the present study were found to be older and more frequently diabetic and hypertensive compared to those who did not require admission. Moreover, underlying chronic heart, lung, and kidney diseases were noted more often in admitted patients than non-admitted patients, which is in agreement with previous studies (Wang et al., 2020; Yang et al., 2020; Ruan et al., 2020; Wu and McGoogan 2020). In addition, the current literature has ample information suggesting that high inflammatory markers can be used as a predictor of worse outcomes in admitted patients with SARS-CoV-2 infection. These include the white blood cell count, absolute lymphocyte count, LDH, CRP, procalcitonin, D-dimer, ferritin, and erythrocyte sedimentation rate (Barrett et al., 2020; Wang et al., 2020). This was also confirmed in the present 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 include 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 cells (CCR−CD45RA+) (TEMRA) (Okada et al., 2008; Romero et al., 2007). The main four subsets of both CD4+ and CD8+ can be further divided into different functional subsets based on the expression of CD27 and CD28 with different cytokine expression (Amyes et al., 2005; Romero et al., 2007; Koch et al., 2008). TEM that are CD27−CD28− are mainly interferon-gamma (IFN-γ) producers (Th1), while CD27−CD28+ TEM are IL-2 (Th0), IFN-γ (Th1), and IL-4 (Th2) producers (Okada et al., 2008)]. TEM and TEMRA T cells are good cytokine producers, including IL-2, IFN-γ, and tumor necrosis factor alpha (TNF-α). Moreover, CD27−CD28− T cells have high effector capability similar to the terminal effector T cells TEMRA subset] (Romero et al., 2007; Koch et al., 2008). Similarly, the combination of PD-1 and CD57 can identify cells with exhausted and or cytotoxic phenotype (Kraaijeveld et al., 2018; Alshekaili et al., 2018).

On the other hand, the naïve T cell is mainly CD27+CD28+ and is a good producer of IL-2, which is required for activation and proliferation (Okada et al., 2008; Koch et al., 2008). CD27+CD28+ naïve T cells are crucial in response to a new virus or vaccine. Those with a reduced frequency of naïve T cells, such as the elderly, are at risk of getting a significant disease when compared to those with plenty of naïve T cells that can respond better to such new viruses (Cunha et al., 2020). This is one of the important explanations for the increased mortality in the elderly after infection with SARS-CoV-2. The present study findings are in line with this: those patients with a low percentage of naïve CD4+ T cells are at a higher risk of mortality.

De Biasi et al. compared the immune system in hospitalized patients with mild to moderate disease ($n = 39$) to that in a healthy uninfected group ($n = 25$). They showed a low count of total CD4+ and CD8+ T cells and their naïve and TCM subsets in the patient group. Moreover, these patients had a higher frequency of cells with the senescent/exhausted phenotype (CD57+PD-1+) (De Biasi et al., 2020). Similarly, we found a reduced percentage of naïve CD4+ and CD8+ T cells and increased exhausted CD4+ and CD8+ T cells in the hospitalized group early in the disease course. In addition, these cells were more cytotoxic compared to the mild non-hospitalized patients in agreement with findings with the other published data (Kusnadi et al., 2021).

Monocytes are the key immune cells and are good producers of inflammatory cytokines like IL-6 (Giavridis et al., 2018; Norelli et al., 2018). They acquire a larger size upon activation and in viral infections (Polilli et al., 2020), including in patients with severe COVID-19 (Lippi and Plebani 2020). Moreover, monocytes can be divided according to the differentiation stage using a combination of CD markers, into immature (CD14+CD16−), differentiated and inflammatory type (CD14+CD16+), and non-classical (CD14−CD16+) (Sánchez-Cerrillo et al., 2020). Examining monocytes in this way has revealed a higher percentage of large inflam-

matory monocytes CD14+CD16+ in hospitalized patients, in line with previous suggestions that patients with severe manifestation have bigger sized monocytes (Khartabil et al., 2020; Lippi and Plebani 2020; Polilli et al., 2020).

In conclusion, the current study identified elevated levels of IL-6, a higher percentage of CD14+CD16+ inflammatory large monocytes, and a 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 SARS-CoV-2 infection. Such predictors could be used for the early identification of patients who might deteriorate and thus need early aggressive interventions. Larger studies are required to validate the current findings, aiming towards better early clinical management.

Declaration of Competing Interest

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

Author contributions

Asma Al Balushi: Conceptualized and 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. Jalila AlShekaili: Reviewed the study design, reviewed the research proposal, supervised receiving blood samples at the immunology laboratory, 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. Mahmood Al Kindi: Reviewed the study design, reviewed the research proposal, supervised receiving blood samples at the immunology laboratory, 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. Zainab Ansari: Field visits, data and sample collection from outpatients and inpatients, and data entry. Murtadha Al-Khabori: Data analysis, writing up results, and manuscript review. Faryal Khamis: Manuscript review and facilitating research work for inpatients. Zaiyana Ambusaidi: Data and sample collection from inpatients and data entry. Afra Al Balushi: Data and sample collection from inpatients and data entry. Aisha Al Huraizi: Data and sample collection from inpatients and data entry. Sumaiya Al Sulaimi: Data and sample collection from inpatients and data entry. Fatma Al Fahdi: Data and sample collection from inpatients and data entry. Iman Al Balushi: Data collection and entry for outpatients and second review of all data. Nenad Pandak: Manuscript review and facilitating research work for inpatients. Tom Fletcher: Overall supervision of the study and manuscript review. Iman Nasr: Proposal review, registered the study, communication with laboratories and companies, manuscript review, and overall workflow and integrity supervision.

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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.

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