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Short and long-term immune changes in different severity groups of COVID-19 disease

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ABSTRACT

Background: There are limited data on short- versus long-term changes in adaptive immune response across different COVID-19 disease severity groups.

Methods: A multicenter prospective study of 140 adult patients with COVID-19 (a total of 325 samples) were analyzed for inflammatory markers and lymphocyte subsets at presentation, week 2, and week 24. **Results:** Inflammatory markers at presentation were higher in the critical/severe than in moderate and mild groups. A predominance of memory B cell response in the mild and moderate group was noted by week 2. In contrast, the immune system in the severe/critical group was dysfunctional, with expansion of exhausted CD8+ T cells and atypical memory B cells. By 24 weeks, there was a possible trend of normalization.

Conclusion: There was substantial difference in the degree of inflammation and distribution of different B and T cell subsets in the different disease severity groups. Despite the initial dysfunctional immune response in the severe/critical group, a comparable memory B and CD8+ T cell responses to the mild group was achieved at 24 weeks.

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Introduction

The spectrum of COVID-19 disease ranges from asymptomatic infection to severe respiratory syndrome with systemic inflammation (Huang et al., 2020b). Patients with severe disease usually have significantly elevated inflammatory markers, such as high C-reactive protein (CRP) and a significant increase in the ferritin and lactate dehydrogenase (LDH) levels compared with mild cases (Chen et al., 2020; Velavan and Meyer, 2020). Moreover, evaluation

of lymphocytes, especially T cells, and subsets can help in assessing the severity and progression of COVID-19 disease, particularly in hospitalized patients (Huang et al., 2020a; Zhang et al., 2020).

There are some recent studies that have looked at different aspects of longevity of immunity to COVID-19 (Dan et al., 2021; Peluso et al., 2021; Shuwa et al., 2021). Some looked at the kinetics of CD4+ T cells, CD8 +T cells, and B cells over 8 months in hospitalized versus nonhospitalized patients (Dan et al., 2021). Memory B cells persisted for 8 months, whereas memory CD4+ (effector memory, TEM, and central memory, TCM) and CD8+ (terminal differentiated effector memory, TEMRA) declined with half-life of ~4 and 6 months (Dan et al., 2021). Another study examined the immunologic differences between different groups (mild, moder-

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ate, and severe) of COVID-19 infection and compared acute versus overall convalescent group (from all initial groups) (Shuwa et al., 2021). It was shown that patients with severe disease had reduced transitional B cells and increased double-negative B cells compared with other groups among other alterations, but all of these have reverted to normal at 6 months after infection (Shuwa et al., 2021). In contrast, the cytotoxic changes among CD8⁺ T cells persisted at 6 months in the convalescent patients (Shuwa et al., 2021). Peluso et al. (2021) has examined immune response in nonhospitalized versus hospitalized patients at 2 months, 4 months, and 8 months. They have shown that specific CD4⁺ and CD8⁺ T cells were detected even at 8 months in both examined groups.

There is limited literature that have looked at short- versus long-term changes in the immune T and B subsets across the different groups of COVID-19 in relation of their inflammatory milieu. Moreover, none of these has examined the long-term immune effect among the different groups to find out if either the mild or severe has developed different memory pattern. Therefore, the aim of this prospective study was to assess changes of T and B subsets in different severity groups of COVID-19 infection at presentation and track their changes at 2 weeks and at 24 weeks after infection.

Methods

Study design

Total of 140 community-acquired COVID-19 cases were recruited in the study in the period between June 1 and November 30, 2020. All patients met the World Health Organization (WHO) case definition, which is a symptomatic patient with a positive SARS-CoV-2 nucleic acid amplification test. Inpatients were all new positive cases getting admitted through the emergency department and recruited after consenting. Outpatients were mainly health care workers or their relatives who came to the family medicine and public health department, who tested positive and consented to participate. All patients recruited from this clinic were mild cases. However, there were six mild cases (using WHO criteria) admitted for different reasons, such as managing dehydration, colitis, bowel ischemia, and a premature rupture of membranes in a pregnant woman. At presentation, all patients were stratified into four groups: mild, moderate, severe, and critical based on WHO classification (Table 1). Serum and whole blood samples were collected for biochemical tests (CRP, LDH, and ferritin) and lymphocyte subsets, respectively.

The tests were performed at presentation (day 0) and at 2 weeks after diagnosis. In addition, other time points were included: on day 7 (moderate category) and on days 3 and 7 (severe and critical categories). Moreover, lymphocyte subset testing was performed for the mild and severe/critical group at 24 weeks after presentation. The data sheet was designed to collect relevant patient's demographic, clinical, laboratory, and radiologic data as presented in Tables 2 and 3.

Laboratory parameters

Cobas s311 analyzer was used to measure CRP, LDH, and ferritin. DuraClone IM T and B cell subsets tubes were used for assessment of different subsets. Used clones for each CD markers are listed in Table 4.

A total of 100,000 events were collected. Briefly, 100 μ l of blood was added to the tube containing the desired cocktail of antibodies and incubated for 20 minutes at room temperature. Then, 100 μ l of lysing solution OptiLyse-B or VersaLyse was added according to the manufacture's (Beckman coulter) recommendation, followed by a wash step. Acquisition of samples and analysis was

done using Navios and Kaluza version 2.1. Gating strategies included are illustrated in Supp. Figure 1 and 2. Some of CD19⁺ B cells and CD4⁺ and CD8⁺ T cells subsets summarized in Table 5.

Statistical analysis

All data were entered and analyzed using SPSS software ver. 22.0 (IBM, Armonk, NY) and GraphPad Prism software v.6.0 (GraphPad Software, San Diego, CA). Group comparisons were performed using Mann-Whitney *U* test (two groups comparisons), Kruskal-Wallis ANOVA test (for more than two groups comparisons) and chi-square test. To adjust for days from onset of symptoms, linear regression analysis was performed. ANOVA was used to analyze the laboratory parameters each time point. Immune cell markers were analyzed using fold changes between different severity groups (moderate/mild, severe and critical/mild, and severe and critical/moderate) and between 2 weeks over baseline in each severity group. *P*-value was adjusted for multiple comparison using false discovery rate (FDR) and data are presented in volcano plot, in which significant hits were identified by fold change of 1.3 and FDR adjusted *P*-value <0.05.

Results

Demographic and clinical characteristics

From 140 adult patients, a total of 325 samples were analyzed for inflammatory markers and 147 samples were assessed for the different distribution of immune subsets.

None were vaccinated against SARS-CoV-2 or previously infected. Of the 140 patients, 54 (38.6%) had mild, 29 (20.7%) had moderate, and 57 (40.7%) had severe/critical disease. Of the severe/critical group, 28 (49.1%) had intensive care unit (ICU) admission and 25 (49%) required mechanical ventilation. On the other hand, one (2%) mild case had ICU admission due to bowel ischemia and one (3.4%) moderate case progressed over time to severe and was admitted to the ICU. A total of 11 patients (19.3%) from the severe/critical group died within 30 days of admission. All patients who survived were discharged before week 8 of their presentation to the hospital. Older age, hypertension, fever, shortness of breath, chest infiltrates, oxygen requirement, acute respiratory distress syndrome, and mechanical ventilation showed statistically significant difference in relation to disease severity in the current study (Tables 3). Of interest, the gastrointestinal manifestations were seen mainly in patients with moderate disease. Steroids and antibiotics were used more in the severe/critical group ($n = 50$ patients, 98%) than the other groups (*P*-value <0.0001) (Table 4). Of note, some of the patients in the different severity groups were on steroids therapy before COVID-19 infection for different indications (e.g., hematologic malignancy, solid organ transplant, and connective tissue disease). Patients with moderate and severe-critical disease received steroids as part of COVID-19 management. On the other hand, mild cases were given short courses of corticosteroids for different clinical indications at the time (asthma, part of induction of a pregnant woman for fetal lung maturation, etc).

Progression of inflammatory markers; CRP, LDH, and ferritin over 2 weeks

At the time of presentation, ferritin and CRP levels were significantly higher in severe/critical than in mild and moderate groups. CRP and ferritin levels follow a downward trend in all groups; levels normalized for the mild group and nearly normalized for the moderate but did not normalize in the severe/critical group at week 2 (Fig. 1). On the other hand, LDH did not differ significantly between the three examined groups throughout the analysis time.

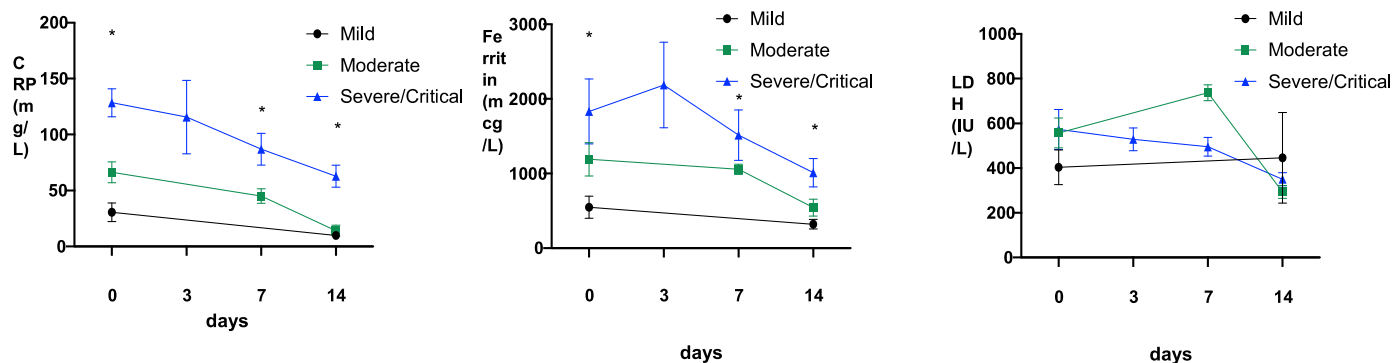


Fig. 1. Levels of CRP ($n = 325$), ferritin ($n = 320$), and LDH ($n = 313$) over the course of 2 weeks after diagnosis of COVID-19 infection among mild ($n = 37$), moderate ($n = 23$), and severe/critical cases ($n = 55$) (mean and SEM are plotted). Reference range: shaded area, CRP 0–8 mg/l, ferritin F: 11–307 μ g/l, M: 24–336 μ g/l, LDH F: 135–214, M: 135–225 IU/l). * P -value < 0.05 linear regression analysis adjusted for days from onset of symptoms.

Frequency of B and T cell subsets among different groups at presentation

We first compared the fold changes of multiple immune cells between disease severity groups. The transitional B cells were 1.4-fold lower in moderate group than the mild group; however, this was not statistically significant (FDR P -value = 0.30) (data not shown). Patients in the severe/critical had lower frequency of transitional B cells than patients in the mild group (fold change 2.2, FDR P -value = 0.02) (Fig. 2B). On the other hand, the moderate had lower frequency of CD27+ CD28- TCM CD8+ T cell subsets than the mild group (Fig. 2A).

On the other hand, moderate group compared with the mild exhibited a significant expansion of B cell responses and higher percentage of circulating total B cells, including total naive (CD19+ CD27-) and total memory (CD19+ CD27+) B cells. The latter included both natural unswitched (CD27+ IgM+ IgD+) and preswitched (CD27+ IgM+ IgD-) memory B cells. In addition, there was an increased frequency of antibody-secreting cells (CD19+ CD27++ CD38++) (Fig. 2B).

Patients in the severe/critical compared with the mild group presented with higher frequency of cytotoxic CD8+ (PD-1+ CD57+) and highly differentiated (cytotoxic and cytokine producers) CD8+ TEMRA (CCR7- CD45RA+) (Fig. 2B).

There was no statistically significant difference in the subset's distributions between the moderate and severe/critical groups (Fig. 2C).

Overall, there was a predominance of memory B cell response in the moderate group, whereas cytotoxic T cells predominated the severe group at the onset of SARS-CoV-2 infection.

Frequency of B and T cell subsets among different groups 2 weeks after infection

At 2 weeks after presentation, lymphocyte subsets showed a lower frequency of transitional B cells and CD27- CD28+ TCM CD4+ T cells in the moderate than the mild group (Fig. 5A). The initial lower frequency of transitional B cells seen at presentation in the severe/critical compared with the mild group was maintained and was even broadened at 2 weeks. (Fig. 3B). The differential expansion of the memory B cell subsets that was seen at presentation between the moderate and mild groups was lost by 2 weeks.

The differential expansion of the cytotoxic and effector CD8+ T cell subsets between the severe/critical and mild groups was lost by 2 weeks. In fact, by 2 weeks, the severe group had more exhausted (PD-1+ CD57-) CD8+ T cells (Fig. 4C). There was no statistically significant difference in the lymphocyte subset's distribu-

tions between the moderate and severe/critical groups (Fig. 4C). Therefore, the main finding seems to be that moderate and severe/critical groups had significant lower transitional B cells at 2 weeks than the mild group.

Short term (2 weeks) immune kinetic after SARS-CoV-2 infection

Expansion of memory B cells and reciprocal contraction of transitional B cells coincide with recovery in the mild and moderate groups. There was an expansion of the total memory B cells (CD19+ CD27+), including both natural unswitched (IgD+ CD27+) and switched (IgD- CD27+) memory B cells. On the other hand, there was a mild downregulation of transitional B cells during the recovery phase compared with the onset of acute infection (Fig. 4A and B).

In addition, patients in the moderate group exhibited a significant expansion of the recent naive B cells (CD19+ CD27-) that have started to lose their surface IgD, including IgM- IgD- and IgM+ IgD- cells. Moreover, there was a reciprocal reduction in total naive B (CD19+ CD27-) and preswitched memory (CD19+ CD27+ IgM+ IgD-) B cells and circulating plasmablast and an extensive reduction in transitional B subsets cells (Fig. 4B).

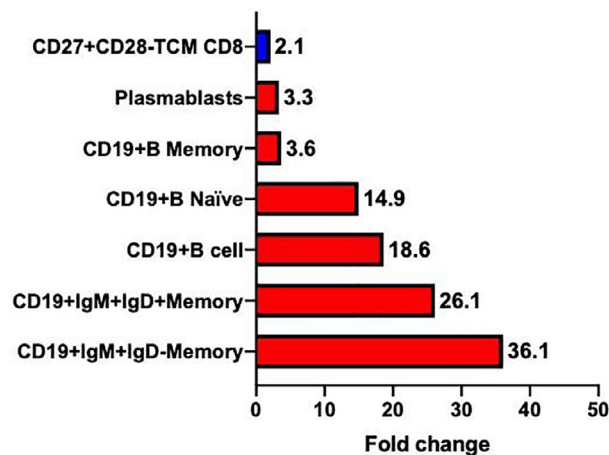
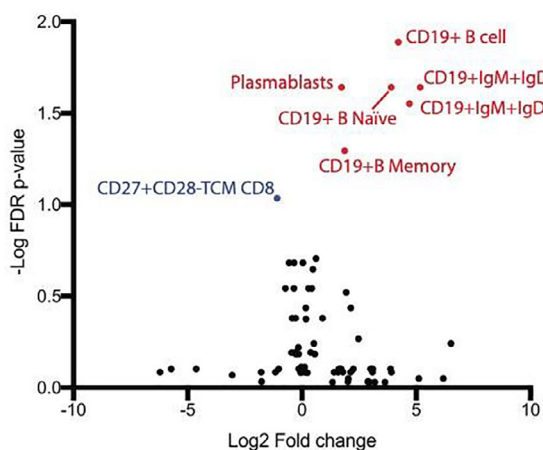
Furthermore, over the course of 2 weeks, there was an expansion of different effector/memory CD8+ T cells subsets, including intermediate effector CD27+ CD28+ TEMRA CD8+ T cells, effector TEM CD8+, TCM CD8+, as well as activated CD45RA- PD-1+ CD8+ T cells. However, this was accompanied with reduction in the highly differentiated effector and cytotoxic CD4+ and CD8+ T cells; highly differentiated effector and cytokine producers (CD27- CD28-) TEM CD4+ and CD8+, cytotoxic (PD-1+ CD57+) CD4+ T and CD8+, and (CD27- CD28+) TCM CD8+ T cells (Fig. 4B).

The short immune kinetic in the severe/critical group was marked with expansion of the intermediate effector CD27+ CD28+ TEMRA CD8+ T cells, CD27+ CD28+ TEM CD8+ T cells, as well as exhausted PD-1+ CD57- CD8+ T cells. In addition, there was some B cell response, including expansion of (IgM+ IgD- naive CD19+ B cell and extrafollicular response, characterized by expansion of atypical memory B cells (CD19+ CD27- IgD-) B cells. Like the immune response in the moderate group, there was a substantial reduction of transitional B cells at 2 weeks compared with presentation (Fig. 4C).

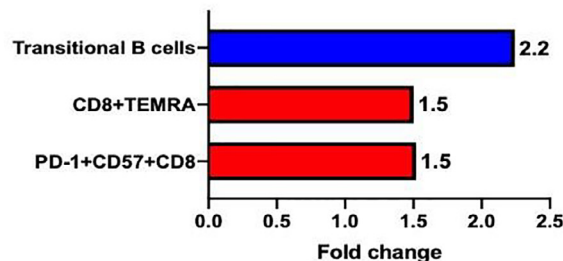
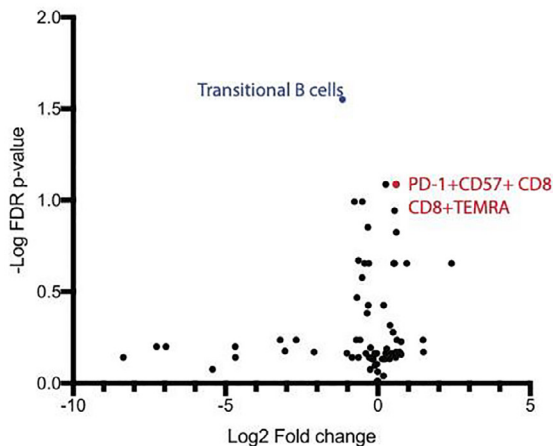
Long-term (24 weeks) immune kinetic after SARS-CoV-2 infection

Examining trends over 24 weeks has focused on subsets with significant fold changes in the 2 weeks follow-up compared with the baseline in the mild and the severe groups. This included nine subsets; memory B cell (CD19+ CD27+), switched

A) Mild Vs Moderate



B) Mild Vs Severe/Critical



C) Moderate Vs Severe/Critical

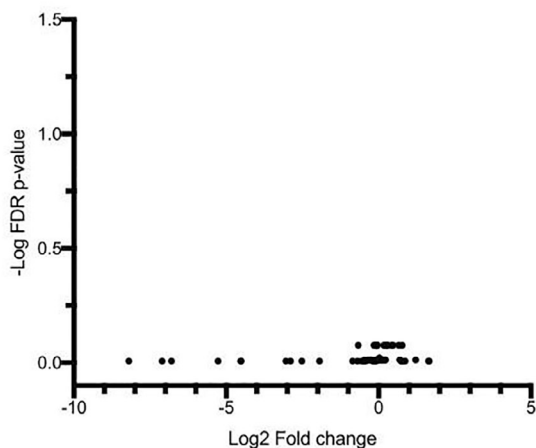
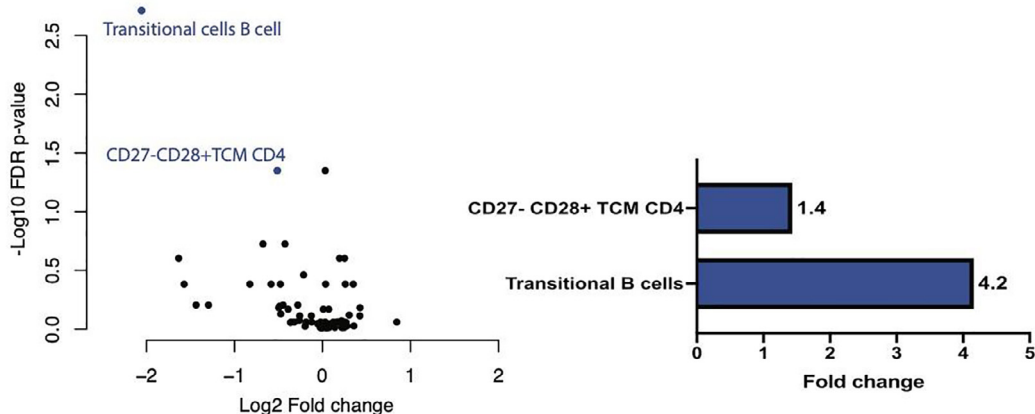
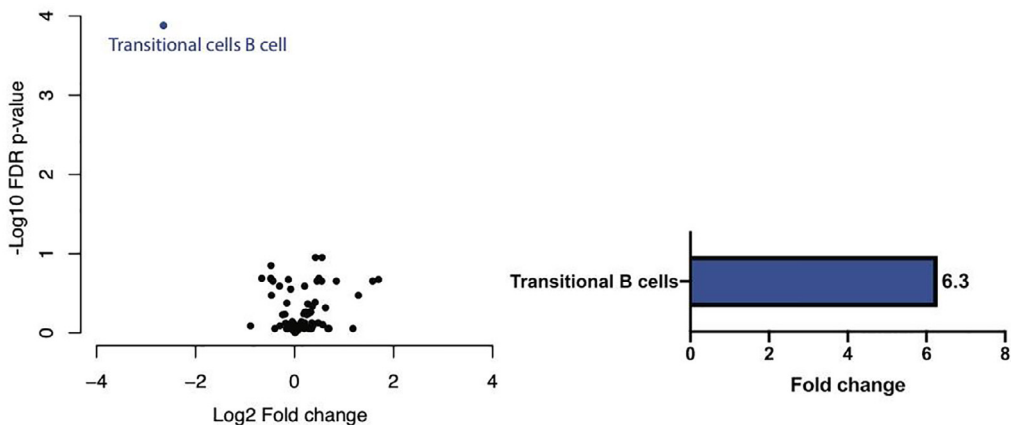


Fig. 2. The differential expansion and contraction of different T and B cell subsets at the time of presentation between; (A) Moderate (n = 18) compared with mild (n = 28), (B) Severe/critical (n = 22) compared with mild, and (C) Severe/critical compared with moderate cases. Percentage of cells were used in the analysis. Significantly expanded subtypes are identified by FDR adjusted P-value <0.05 (Mann-Whitney U test) and fold change of above 1.3, in which red labeled subtypes are significantly upregulated and blue ones are significantly downregulated. Blue bars: downregulation fold change values, red bars: upregulation fold change values. Cell details: CD27+ CD28- out of TCM (CD197+ CD45RA-) CD8+ Tcell; Plasmablast (CD27+ CD38+) out of CD19+ B cell; B memory (CD19+ CD27+) out of CD45+ leucocyte cell; B naive (CD19+ CD27-) out of CD45+ leucocyte cell; B cell (CD19+) out of CD45+ leucocyte cell; IgM+ IgD+ out of memory (CD27+ CD19+) B cell; IgM+ IgD- out of memory (CD27+ CD19+) B cell; Transitional (CD24+ CD38+) B cell out of CD19+ B cell; TEMRA (CD197- CD45RA+) out of CD8+ T cell and PD-1+ CD57+ out CD8 T cell.

A) Mild Vs Moderate



B) Mild Vs Severe/Critical



C) Moderate Vs Severe/Critical

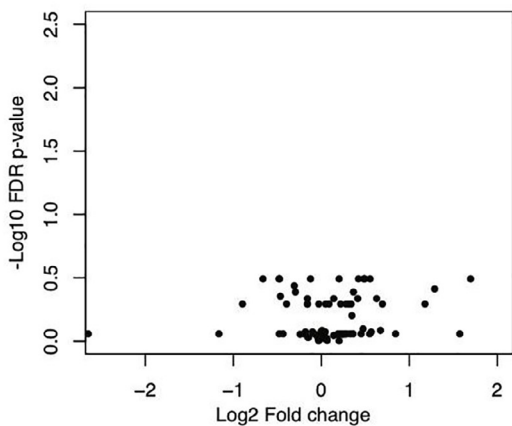


Fig. 3. The differential expansion and contraction of different T and B cell subsets at 2 weeks between; (A) Moderate (n = 18) compared with mild (n = 28), (B) Severe/critical (n = 22) compared with mild, and (C) Severe/critical compared with moderate cases. Percentage of cells were used for analysis. Significantly expanded subtypes are identified by FDR adjusted *P*-value <0.05 (Mann-Whitney *U* test) and fold change of above 1.3, in which blue labeled subtypes are significantly downregulated. Blue bars: downregulation fold change values. Cell details: CD27- CD28+ out of TCM (CD197+ CD45RA-) CD4+ Tcell; Transitional (CD24+ CD38+) B cell out of CD19+ B cell.

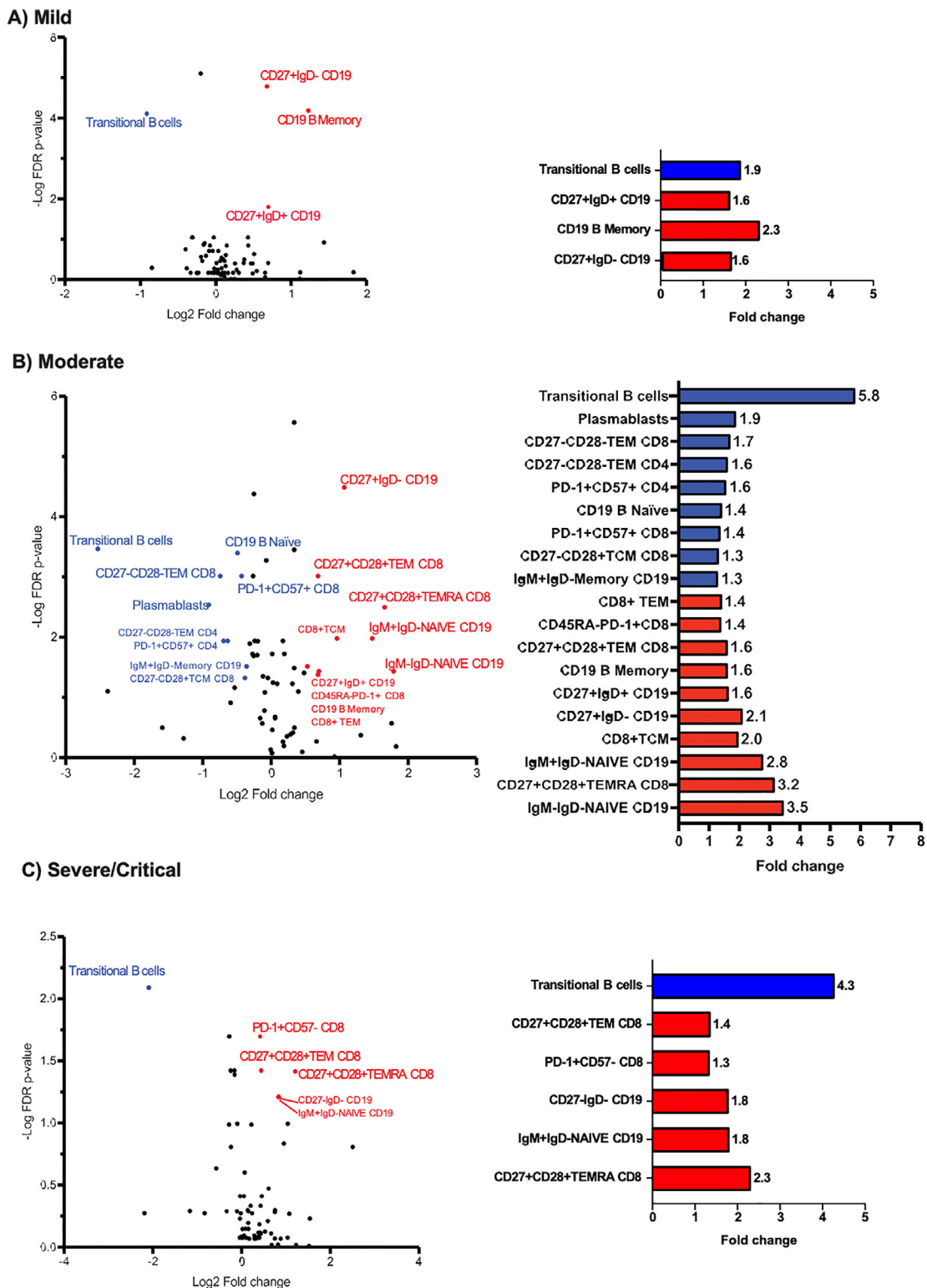


Fig. 4. The differential immune progression of different T and B cell subsets over 2 weeks; A-C, each comparison was made between 2 weeks over basal levels. (A) Mild (n = 28), (B) Moderate (n = 18), and (C) Severe/critical (n = 22) cases. Percentage of cells were used in the analysis. Significantly expanded subtypes are identified by FDR adjusted P-value <0.05(Mann-Whitney U test) and fold change of above 1.3, in which red labeled subtypes are significantly upregulated and blue ones are significantly downregulated. Blue bars: downregulation fold change values, red bars: upregulation fold change values. Cell details: Transitional (CD24+ CD38+) B cell out of CD19+ B cell; Plasmablast (CD27+ CD38+) out of CD19+ B cell; B memory (CD19+ CD27+) out of CD45+ leucocyte cell.; CD27+ IgD- out of B (CD19+) cell; CD27- CD28- out of TEM (CD197- CD45RA-) CD8+ T cell; CD27- CD28- out of TEM (CD197 CD45RA-) CD4+ T cell; PD-1+ CD57+ out CD4 T cell; B naive (CD19+ CD27-) out of CD45+ leucocyte cell; PD-1+ CD57+ out CD8 T cell; CD27- CD28+ out of TCM (CD197+ CD45RA-) CD8+ T cell; IgM+ IgD- out of memory (CD27+ CD19+) B cell.; TEM (CD197- CD45RA-) out of CD8+ T cell; CD45RA- PD-1+ out of CD8+ T cell; CD27- CD28+ out of TCM (CD197+ CD45RA-) CD8+ T cell; CD27+ IgD+ out of B (CD19+) cell; TCM (CD197+ CD45RA-) CD8+ T cell; IgM+ IgD- out of naive (CD27- CD19+) B cell; CD27+ CD28+ out of TEMRA (CD197- CD45RA+) CD8+ T cell; IgM- IgD- out of naive (CD27- CD19+) B cell; CD27+ CD28+ out of TEM (CD197- CD45A-) CD8+ T cell; PD1+ CD57- out CD8 T cell; CD27- IgD- out of B (CD19+) cell and CD27+ CD28+ out of TEMRA CD197- CD45RA+) CD8+ T cell.

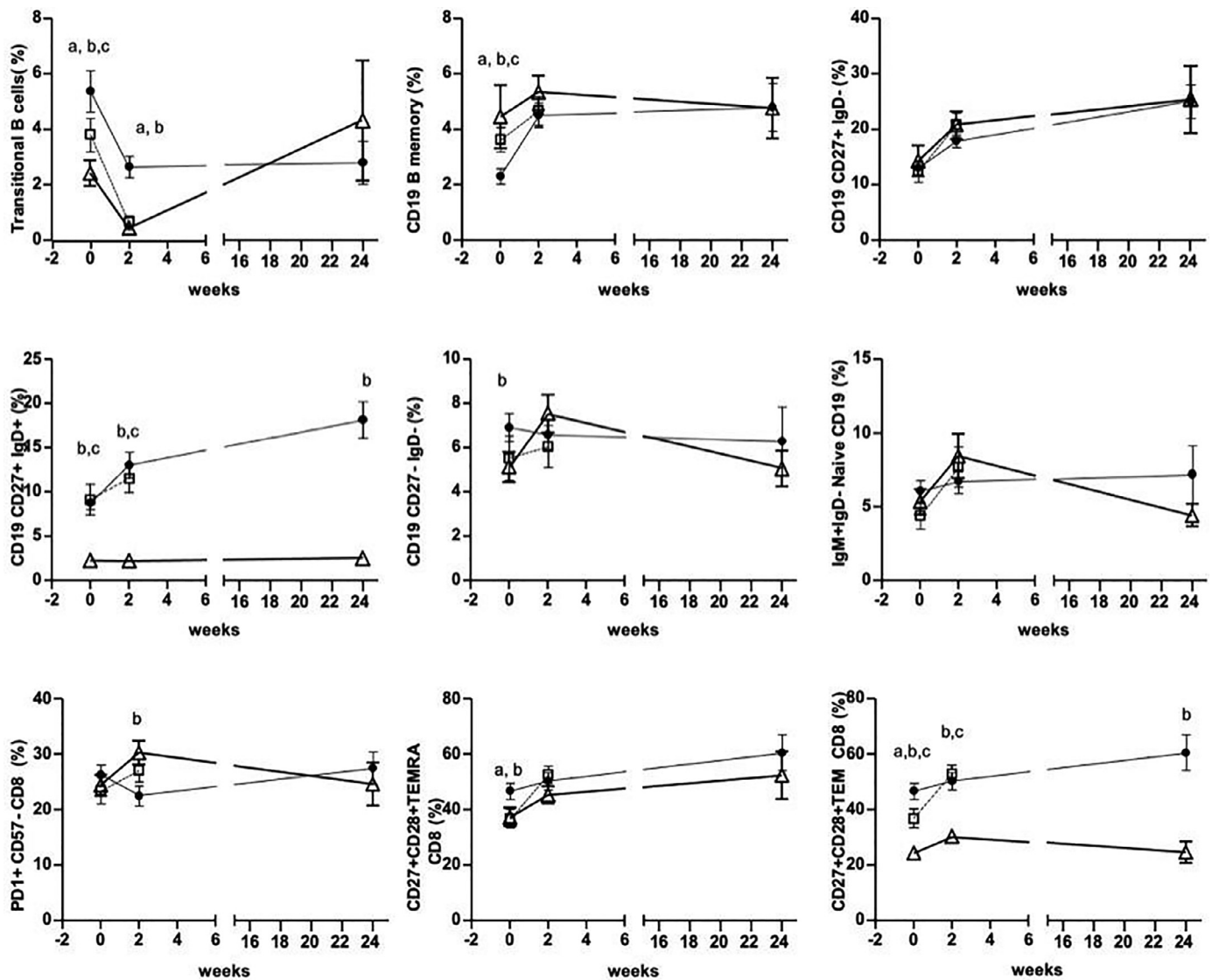


Fig. 5. The differential immune progression of different T and B cell subsets at time; 0, 2, and 24 weeks for the different groups; mild, moderate and severe/critical (unfilled triangle) patients with COVID-19. Plotting only those with significant fold changes from Fig. 4 A and C. Mild (dark filled circle) at 0 and 2 (n = 28) and (n = 5) at 24 weeks. Moderate (unfilled square) at 0 and 2 (n = 18). Severe (unfilled triangle) at 0 and 2 (n = 22) and (n = 4) at 24 weeks. Data shown are mean and SEM for percentage of cells from flow cytometry. Significant *P*-values <0.05 (Mann-Whitney *U* test) between groups at each time point indicated as a, b, and c: a: between mild and moderate, b: between mild and severe, c: between moderate and severe. Cell details: Transitional (CD24+ CD38+) B cell out of CD19+ B cell; B memory (CD19+ CD27+) out of CD45+ leucocyte cell; CD27+ IgD- out of B (CD19+) cell; CD27+ IgD+ out of B (CD19+) cell; CD27- IgD- out of B (CD19+) cell; IgM+ IgD- out of naive (CD27- CD19+) B cell; PD-1+ CD57-out CD8 T cell; CD27+ CD28+ out of TEMRA (CD197- CD45RA+) CD8+ T cell and CD27+ CD28+ out of TEM (CD197- CD45RA-) CD8+ T cell.

memory B cell (CD19+CD27+IgD-), unswitched memory B cell (CD19+CD27+IgD+), transitional B cell (CD19+ CD38+ CD24+), IgM+ IgD- naive B cell (CD19+CD27-), and double-negative memory (CD19+ CD27- IgD-) B cell. It also included CD27+ CD28+ TEM CD8+ T cells, CD27+ CD28+ TEMRA CD8+ T cells, as well as exhausted CD8+ T cells (PD-1+ CD57-) (Fig. 4 A and C). Despite the lack of significance owing to the small sample size (nine in both groups) in the 24 weeks follow-up, there was an observed trend in most of these subsets.

In the mild group, the significant increasing trend seen at 2 weeks among different memory B subsets; total (CD19+ CD27+), unswitched (CD19+ CD27+ IgD+), and switched (CD19+ CD27+ IgD-) has persisted at 24 weeks after SARS-COV-2 infection. In contrast, the decreasing trend in the transitional B cells seen initially at 2 weeks has plateaued at 24 weeks (Fig. 5).

In the severe/critical group, after initial drop in the frequency of circulating transitional B cells, there was an expansion of tran-

sitional B cells, with a reciprocal contraction of IgM+IgD- naive B cells during the time from 2 to 24 weeks. Moreover at 24 weeks, (CD27+CD28+ TEMRA) CD8+ T cells continued to increase, whereas (CD27+CD28+ TEM) CD8+ T has decreased back to the baseline level. These changes were accompanied with the resolution of the exhausted CD8+ T cells and atypical memory B cell subsets (Fig. 5).

Discussion

Our study demonstrated significant alterations in the frequency of different lymphocyte subsets at the different assessed time points among the examined COVID-19 disease groups. Patients with mild disease had higher transitional B cells than patients in moderate and severe groups. The significance of these transitional B cells is still controversial, but more evidence is accumulating to support their role in the robust humoral response against vi-

ral infection and production of neutralizing antibodies. This role is supported in recent reports, where higher frequency of transitional B cells was reported in patients with mild COVID-19 disease (Oliviero et al., 2020; Sosa-Hernández et al., 2020).

B cell response in patients with moderate disease at the time of recruitment showed massive memory B cells responses compared with the mild group consisting of germinal center derived B cells, including unswitched (CD19+ CD27+ IgM+ IgD+) memory B and preswitched (CD19+ CD27+ IgM+ IgD-) (Turner et al., 2021), total (CD19+CD27+) memory B (Oliviero et al., 2020), and plasmablast cells. This could be explained knowing that blood was collected on a median of 2 days after symptom onset for the mild group and 5 days for the moderate (Auladell et al., 2019). The outstanding expansion of memory B cell subsets seen at recruitment in the moderate group compared with the mild group was not sustained at 2 weeks. This might suggest that the mild group had expanded their memory B cell subsets to an equal level (Fig. 5) (Sosa-Hernández et al., 2020). In fact, this increase of the switched and unswitched memory B cells is known to be seen in patients with shorter duration of symptoms (Dan et al., 2021; Newell et al., 2021).

The origin of these newly formed memory B cell subsets could be from recruitment of immature transitional B cells and or mature naive B cells (Zhou et al., 2020) that are differentiated into different memory subsets and plasma cells (Bemark, 2015; Carrion et al., 2019), explaining the reciprocal downregulation of these cells. In contrast to the initially seen upregulation, there was a contraction of plasmablasts in the moderate group in the 2 weeks follow-up (Fig. 5B) and this was thought to be in association with generation of germinal center-derived long-lived plasma cells (Newell et al., 2021). The expansion of different memory B cell subsets, including total, unswitched, and switched B memory subsets, persisted at 6 months after infection is suggestive of a likely long-lasting immunity in examined groups in agreement with previous studies (Dan et al., 2021).

Atypical memory B cells seen in the severe/critical group was reported previously (Oliviero et al., 2020; Woodruff et al., 2020) and found to be associated with reciprocal disappearance of transitional and naive B cells, suggesting that they could be the origin of atypical memory B cells. They are seen usually in inflammatory diseases, especially chronic diseases, such as systemic lupus erythematosus, among others (Ruschil et al., 2020; You et al., 2020). It is reported to be associated with increase in the levels of inflammatory markers, such as CRP, and production of high levels of neutralizing antibodies but not sufficient to control the disease (Woodruff et al., 2020). In fact, it was documented that severe group had higher level of SARS-CoV-2-specific antibodies that are not sufficient to compact the high inflammatory response (Shrock et al., 2020). Despite this initial formation of atypical memory B cell in the severe group, there was expansion of the total and switched memory B cells to a comparable level to the mild at 24 weeks.

After initial antigen exposure and activation, naive CD8+ T cells can differentiate into effector or central memory phenotype. There are different models to explain the pathway toward differentiation of different memory subsets. One of them is the presence of high antigen dose, coupled with inflammation (Joshi and Kaech, 2008; Kinjyo et al., 2015). High inflammatory milieu and high antigen dose favors the development of effector and cytotoxic CD8+ T cells, such as TEM and TEMRA (Rao et al., 2020), whereas lower dose of antigen and less inflammation direct formation of TCM CD8+ T cells (Joshi and Kaech, 2008).

The mild group had higher frequency of circulating effector CD8+ T cells, such as TEM and TEMRA (Fig. 5), than the severe group, in line with the important role of T cell response in the control of the infection in the mild group (Steiner et al., 2021). Of

note, all observed fold changes over the 2 weeks in the mild group are not massive (all fold changes are less than five), which might suggest the prompt initial immune response was enough to handle the infection. These immune responses might explain the mild symptoms and prompt resolution of inflammatory parameters in line with previous findings (Bergamaschi et al., 2021).

Similarly, the moderate group had progressive increase in the TCM CD8+ T cells (not statistically significant, data not shown) as well as progressive expansion of intermediate effector CD27+ CD28+ TEMRA CD8 SARS-CoV-2-specific T cells that were efficient in handling SARS-CoV-2 infection, as described previously (Dan et al., 2021; Neidleman et al., 2020). This response might have contributed to the control and dampening response of the highly differentiated effector and cytotoxic (CD27- CD28- TEM) CD4+ and CD8+ T cells in this group.

Inflammatory markers, including CRP, ferritin, and LDH, at presentation were higher in the critical/severe group than in moderate and mild groups. CRP and ferritin have normalized for the mild and moderate but not completely in the severe/critical group at week 2 (Fig. 1).

Possibly due to the prolonged inflammation, the severe/critical group had initial significant expansion of effector (TEMRA) and cytotoxic (CD57+ PD-1+) CD8+ T cells (Gong et al., 2020; Zheng et al., 2020) that was not able to control the infection, leading to more inflammation. This has progressed during the 2 weeks into dysfunctional immune response with exhausted CD8+T cell (PD-1+ CD57-) (Haring et al., 2006; Rha and Shin, 2021; Tilstra et al., 2018) and atypical memory B cell formation (De Biasi et al., 2020; Ruschil et al., 2020; Zheng et al., 2020), rather than proper and typical memory B cells. At 24 weeks after SARS-CoV-2 infection, immune response has eventually recovered as frequency of atypical memory B cell and exhausted CD8+ T cell went back to their baseline level. Moreover, there was increase of the effector and cytotoxic (C27+ CD28+) TEMRA CD8 +T cells and different memory B cell subsets.

Our study had limitations. First, loss of follow-up sampling, especially at 24 weeks. Second, sequencing of the circulating variants was not available during the study; therefore, it is unclear if this is replicative with other variants. For future studies, it will be interesting to examine the immune kinetics in vaccinated versus non-vaccinated groups, presenting with different severity of COVID-19 infection and replicate these finding on a bigger cohort.

Conclusion

There was substantial difference in the degree of inflammation and distribution of different B and T cell subsets in the different disease severity groups. A good memory B and effector CD8+ T cell responses were formed in the mild and moderate groups that have persisted until 24 weeks after presentation. Despite the initial dysfunctional immune response in the severe/critical group, possibly due to the higher inflammatory milieu, there were comparable memory B and effector CD8+ T cell responses at 24 weeks with the mild group.

Conflict of interest

The authors have no competing interests to declare.

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Ethical approval

Ethical approval was obtained through the institutional research committees (MREC #212 and AFMS-MREC 010/2020).

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:[10.1016/j.ijid.2022.07.026](https://doi.org/10.1016/j.ijid.2022.07.026).

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