IMMUNOLOGICAL RESPONSE IN COVID-19 POSITIVE PATIENTS: CASE SERIES

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ABSTRACT

This is a case series involving eight positive COVID-19 patients in different clinical stages, highlighting their immunological response assessed through lymphocyte subset enumeration and serum immunoglobulin using flow cytometry and turbidimetry, respectively. The aim of this study is to determine the role of lymphocytes and immunoglobulins in relation to the clinical outcome of COVID-19 infection. The majority of the patients (75%) showed a significant reduction in the total T lymphocytes and CD8+ T lymphocytes. In contrast, only one patient showed a reduction in total B lymphocytes and NK cells. The serum immunoglobulin levels were unremarkable. The CD8+ T cell lymphopenia was a notable feature that was potentiated to be a significant clinical outcome predictor for COVID-19 infection.

KEYWORDS: COVID-19, SARS-CoV-2, Lymphopenia, Immune Response, CD8+ T Cells

INTRODUCTION

A novel coronavirus, named SARS-CoV-2 has caused a cluster of cases of pneumonia in Wuhan, China in December 2019. It has rapidly spread globally and the World Health Organization (WHO) declared it as a pandemic on March 11, 2020. This highly virulent virus has already claimed more than 5 million lives, with affected cases exceeding 200 million worldwide. SARS-CoV-2 is acquired by exposure to respiratory droplets which are expelled by an infected individual. It presents a wide range of clinical manifestations, ranging from asymptomatic to severe pneumonia or even death. The virus replicates in the lung epithelium, resulting in pneumonia. It was noted that the elderly was more prone to severe infections and their mortality rate increased with age (1).

The host immune response plays a vital role in defence against this infection. An elevated total T cell count, including $CD4^+$ and $CD8^+$ T cells, has been shown to be a predictor of a less severe illness with a better outcome (2). There is evidence that lymphopenia and elevated cytokines lead to disease progression and even death in critically ill patients (3). Infection with SARS-CoV-2 can stimulate epithelial-cell-mediated production of reactive oxygen species (ROS) causing cell death. ROS can also stimulate the synthesis of (NOD)-like receptor protein 3 inflammasome (NLRP3) and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) which contribute to increased cytokine levels and therefore lead to cytokine storm. This essentially causes immune invasion, which can lead to tissue damage, multi-organ failure and death (4).

Case Report

This is a case series of eight SARS-CoV-2 positive patients admitted to Hospital Sungai Buloh, which is one of the referral hospitals for COVID-19 in Malaysia. The gold standard RT-PCR method was used to diagnose these cases. Clinically, they are classified into 5 categories ranging from asymptomatic to critical. The critical stage is defined as respiratory failure, septic shock and/ or multi-organ failure developing in an infected individual. Table 1 shows various clinical stages of SARS-CoV-2 infection. All of them are adult patients, with the majority being males. The lymphocyte subset enumeration test was performed using the BD FACS CantoII flow cytometer with a commercial kit from BD Biosciences, USA (BD Multitest™ CD3/CD8/CD45/CD4 and CD3/CD16+CD56/CD45/CD19) while the serum immunoglobulin (Ig) was performed by the turbidimetry method using SPAPlus (Binding Site) measuring IgG, IgA and IgM. Table 2 depicts the demographics, lymphocyte subset enumeration and serum immunoglobulin levels. Figures 1 and 2 illustrate the dot plot distribution for lymphocyte subsets and serum immunoglobulins, respectively.

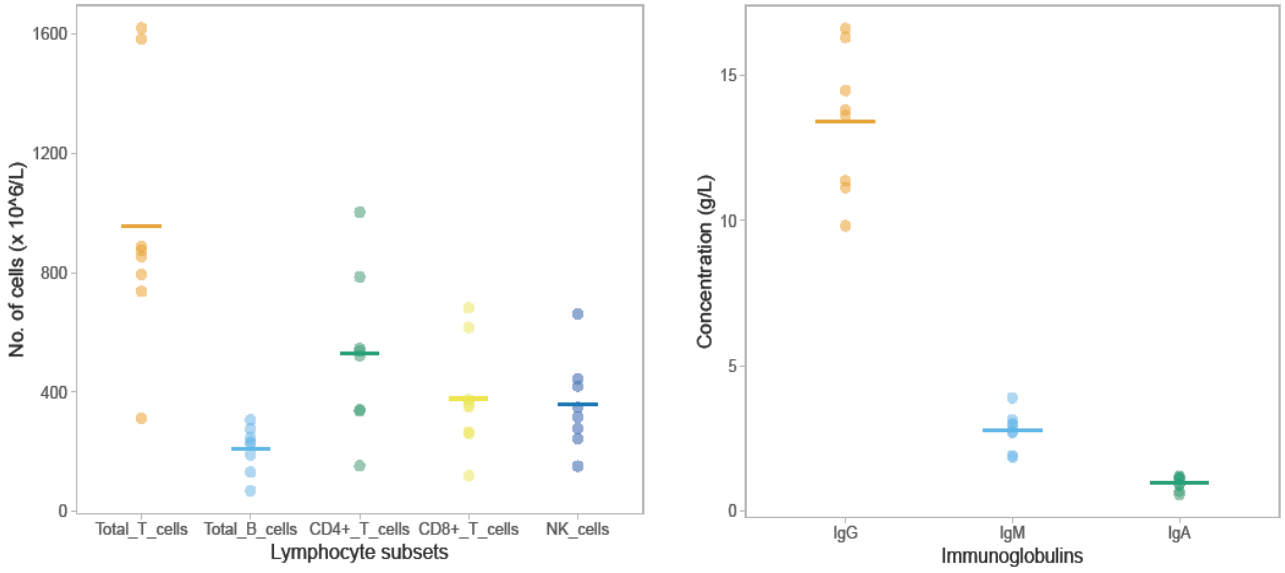

Figure 1. Dot plots of Lymphocyte Subsets (left) and Immunoglobulin (right) of Patients.

Table 1. Clinical category and criteria of SARS-CoV-2 infection.

Clinical categories	Criteria
Asymptomatic or Pre-symptomatic illness	SARS-CoV-2 positive individual (by nucleic acid amplification test or antigen test) but without the symptoms that are consistent with COVID-19.
Mild illness	Individuals with various signs and symptoms of COVID-19such as fever, cough, sore throat, headache, myalgia, loss of smell and taste sensation, malaise, vomiting, diarrhoea, nausea but without shortness of breath, dyspnoea and abnormal chest imaging.
Moderate illness	Individuals with evidence of lower respiratory tract disease (clinically or imaging) but with oxygen saturation, SpO_2 ≥94% on room air at sea level.
Severe illness	Individuals with SpO_2 ≤94% on room air at sea level, respiratory rate ≥30 breaths/min, a ratio of arterial partial pressure of oxygen to fraction of inspired oxygen (PaO_2 / FiO_2) <300 mm Hg or lung infiltrates >50%.
Critical illness	Individuals with respiratory failure, septic shock and/or multi-organ dysfunction.

Adapted from 'COVID-19 Treatment Guidelines: Clinical spectrum of SARS-CoV-2 infection, NIH, USA'.

Critical cases

Cases 1, 2 and 3 are the three critical stage patients, with all of them showing total T cell and CD8+ T cell lymphopenia. Case 1 involves a 61-year-old male with an underlying coronary artery disease who eventually progressed to severe pneumonia with acute respiratory distress syndrome (ARDS) and acute kidney injury (AKI). He subsequently required ventilator support and ICU care. In addition, his NK cells and CD4+ T cells were also reduced. Case 2 is a 52-year-old male with underlying hypertension and bronchial asthma. He too developed ARDS and AKI, which required ventilator support and ICU care. In addition to the total T cell and CD8+ T cell lymphopenia, he also showed CD4+ T cell lymphopenia. Case 3 is a 43-year-old man with obesity (BMI >35) and hypertension. Similar to the other two cases, he developed ARDS and AKI and also required ventilator support.

Non-critical cases

Cases 4 through 8 are non-critical cases. These ranges from mild to severe illnesses. Cases 5, 6 and 8 showed total T cell and CD8+ T cell lymphopenia. In addition, case 5 showed a reduction in CD4+ T cells and total B cells. Cases 4 and 7 did not show any deranged lymphocyte subset enumeration. Although case 8 has an underlying diabetes mellitus (DM), he is not on any oral or injectable hypoglycaemic medications and depends solely on dietary control. Nevertheless, this patient progressed to a severe illness category. The rest of the cases in this group only experienced mild to moderate illnesses.

Serum immunoglobulins

The serum immunoglobulins of all these patients were unremarkable. Only case 4 and case 7 showed a mild elevation in the serum IgG levels.

A rapid and early host immune response is vital in controlling the spread and progression of the COVID-19 illness. There could be several factors that determine the immune response and disease severity. Old age and male sex are associated with an increased risk of COVID-19 complications, whereas females mount stronger T cell activation after being infected with SARS-CoV-2 (5). This is in agreement with our study as the critical cases are all males with some underlying co-morbidities and the only female, which is case 7, did not show any derangements in the lymphocyte subsets.

Studies have reported that the incubation period for SARS-CoV-2 infection is between 4 to 7 days before the onset of symptoms, and it may take another 7 to 10 days for it to progress to severe or critical stages (6). In general, this is the time taken for host T cells to elaborate on their response to control the virus, which correlates with the time taken for the patients to recover or progress to severe illness. It is within this time frame that a strong initial T cell response may be protective, whereas an inadequate T cell response may contribute to severe outcomes (7).

The body's antiviral response depends on the interaction between antigen-presenting cells (APC) and an antigen when a virus enters the cells. The infected cells are recognised by cytotoxic T lymphocytes through viral peptides on the cell surface, ultimately leading to apoptosis. Another role played by the cytotoxic T lymphocytes is the secretion of various cytokines that contribute to the host defence (8). In critical stages, it is observed that lymphopenia, particularly of $CD8^+$ T cells, occurs. Chen (9) demonstrated a decrease in absolute

Table 2. Demographics, lymphocyte subset enumeration and serum immunoglobulin

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7	Case 8
Age (years)	61	52	43	27	44	59	35	61
Sex	Male	Male	Male	Male	Male	Male	Female	Male
Co-morbidity	CAD	HPT BA	HPT Obesity	Nil	Nil	Nil	Nil	DM
Lymphocyte subset enumeration (x106/L)								
i) Total T Cells	313	737	875	1584	795	853	1620	888
ii) Total B Cells	248	233	132	307	69	190	277	227
iii) CD4+ T cells	153	341	538	786	337	546	1003	522
iv) CD8+ T cells	120	261	375	617	356	351	682	266
v) NK cells	152	279	663	317	349	444	420	245
Serum immunoglobulins (g/L)								
i) IgG	13.62	11.14	9.82	16.62	11.37	13.82	16.31	14.49
ii) IgM	2.95	1.91	2.70	2.73	1.85	3.89	2.99	3.14
iii) IgA	1.19	1.57	1.13	0.91	1.08	0.89	0.68	1.16

CAD: Coronary artery disease, HPT: Hypertension, BA: Bronchial asthma, DM: Diabetes mellitus
Normal range: (I) Lymphocyte subsets a) Total T cells: 988-3,912; b) Total B cells: 130-716; c) CD4+ cells: 431-1,976 d) CD8+ cells: 385-1,805; e) NK cells: 227-1,354 (II) Serum immunoglobulins a) IgG: 7.0-16.0; b) IgA: 0.70-4.0; c) IgM: 0.40-2.3.

total T cell counts as well as $CD8^+$ T cells in almost all their COVID-19 infected patients, and this was more pronounced in the severe cases. In this case series, 6 patients (75%), showed a reduction in absolute total T cell and $CD8^+$ T cell counts, with a mean value of 958.1 and 378.5, respectively. This highlights the similarity with the previous study.

As for CD4+ T cells, some studies demonstrated that there was a lack of $CD4^+$ T cell response during SARS-CoV-2 infection (10), which is usually seen in chronic infection and not in acute viral infection (11). On the contrary, others have suggested over activation of the $CD4^+$ T cells during COVID-19. Only 3 patients (37.5%) showed a reduction in the absolute $CD4^+$ T cell counts in this case series.

Tayakolpour et. al (2) suggested that the reason for the lymphopenia could be attributed to the inflammatory cytokine storm, whereby pro-inflammatory cytokines, such as IL-6 and TNF-α are related to lymphopenia. A study by Diao (13) found that SARS-CoV-2 infection can cause an increased cell surface expression of programmed cell death protein 1 (PD-1) and T cell immunoglobulin and mucin domain 3 (Tim-3), which correlates with disease severity. These markers suggest that the development of lymphopenia could result from T cell exhaustion.

A study conducted by Adamo *et al*. found that there is substantial global T cell apoptosis, especially among CD8+ T cells, which could be caused by the inflammatory microenvironment initiated by SARS-CoV-2 infection. The increase of T cell apoptosis in critical illness could be central to the development of lymphopenia and, is closely associated with a highly inflammatory innate immune response (14). This hyper-activated innate immune response could possibly be one of the mechanisms for the occurrence of cytokine storms in COVID-19 infection (3).

The function of B cells in the initial immune response remains to be explored. However, it may not be a key player in the recovery process as it has been reported that two X-linked agammaglobulinemia (XLA) patients infected with SARS-CoV-2 have recovered completely (15). It was found that only 1 patient (12.5%) had decreased absolute total B cells, which suggests the limited role of B cells during acute infection.

In conclusion, it is notable that T cells, in particular, $CD8^+$ T cell lymphopenia is associated with severe cases of SARS-CoV-2 infection, although the main factor contributing to this still need to be elucidated. The main limitation of this study is that it is not supported by statistical findings, as in any case reports. An extensive study on the serial assessment of $CD4^+$ and $CD8^+$ T cells at various clinical stages, along with related cytokine levels, will perhaps provide greater insight into the immunopathology of SARS-CoV-2 infection in the near future.

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REFERENCES

1. Westmeier J, Paniskaki K, Karaköse Z, Werner T, Sutter K, Dolff S, *et al.* Impaired cytotoxic CD8+ T cell response in elderly COVID-19patients. ASM Journals mBio. 2020;11(5):e02243-20. doi.org/10.1128/mBio.02243-20.
2. Tavakolpour S, Rakhshandehroo T, Wei EX, Rashidian M. Lymphopenia during the COVID-19infection: what it shows and what can be learned. Immunol Lett. 2020; 225: 31-2. doi.org/10.1016/j.imlet.2020.06.013
3. Tang L, Yin Z, Hu Y, Mei H. Controlling Cytokine Storm Is Vital in COVID-19. Front. Immunol. 2020; 11:570993. doi.org/10.3389/fimmu.2020.570993.
4. Bhaskar S, Sinha A, Banach M, Mittoo S, Weissert R, Kass JS, et al. Cytokine Storm in COVID-19-Immunopathological Mechanisms, Clinical Considerations, and Therapeutic Approaches: The REPROGRAM Consortium Position Paper. Front. Immunol. 2020; 11:1648. doi.org/10.3389/fimmu.2020.01648.
5. Takahashi T, Ellingson M.K, Wong P, Israelow B, Lucas C, Klein J, *et al.* Sex differences in immune responses that underlie COVID-19disease outcomes. Nature. 2020; 588, 315–320. doi.org/10.1038/s41586-020-2700-3.
6. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, *et al.* Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet. 2020; 395(10223): 497-506. doi.org/10.1016/s0140-6736(20)30183-5
7. Peng Y, Mentzer AJ, Liu G, Yao X, Dong D, Dejnirattisai W, *et al*. Broad and strong memory CD4+ and CD8+ T cells induced by SARS-CoV-2 in UK convalescent individuals following COVID-19. Nat Immunol. 2020; 21,1336-1345. doi.org/10.1038/s41590-020-0782-6
8. Janeway CA Jr, Travers P, Walport M, Shlomchik MJ. Immunobiology: The Immune System in Health and Disease. 5th edition. New York: Garland Science; 2001. T cell-mediated cytotoxicity. Available from: https://www.ncbi.nlm.nih.gov/books/NBK27101/
9. Chen G, Wu D, Guo W, Cao Y, Huang D, Wang H, *et al.* Clinical and immunological features of severe and moderate Coronavirus Disease 2019. J Clin Invest. 2020; 130:2620–9. doi:10.1172/JCI137244
10. Zhou Y, Zhang Z, Tian J, Xiong S. Risk factors associated with disease progression in a cohort of patients infected with the 2019 novel coronavirus. Ann Palliat Med. 2020; 9(2):428–436. doi.org/10.21037/apm.2020.03.26
11. Hasenkrug KJ, Chougnet CA, Dittmer U. Regulatory T cells in retroviral infections. PLoS Pathog. 2018; 14:e1006776. doi.org/10.1371/journal.ppat.1006776.
12. Mathew D, Giles JR, Baxter AE, Oldridge DA, Greenplate AR, Wu JE, *et al.* Deep immune profiling of COVID-19patients reveals distinct immunotypes with therapeutic implications. Science. 2020;369(6508): eabc8511. doi.org/10.1126/science.abc8511
13. Diao B, Wang C, Tan Y, Chen X, Liu Y, Ning L, *et al.* Reduction and functional exhaustion of T cells in patients with coronavirus disease 2019 (COVID-19) Front. Immunol. 2020;11(827). doi.org/10.3389/fimmu.2020.00827
14. Adamo S, Chevrier S, Cervia C, Zurbuchen Y, Raeber ME, Yang L, *et al.* Profound dysregulation of T cell homeostasis and function in patients with severe COVID-19. Allergy. 2021;76(9):2866-2881. doi.org/10.1111/all.14866.
15. Soresina A, Moratto D, Chiarini M, Paolillo C, Baresi G, Foca E, et al. Two X-linked agammaglobulinemia patients develop pneumonia as COVID-19 manifestation but recover. Pediatr. Allergy Immunol. 2020;31(5):565-69. doi.org/10.1111/pai.13263.