RESEARCH / ARAŞTIRMA
DOI: 10.26650/IUITFD.1079629
İst Tıp Fak Derg 2022 / J Ist Faculty Med 2022

A FLOW CYTOMETRIC EVALUATION OF B LYMPHOCYTE CELLS AND SUBGROUPS OF CHILDREN DIAGNOSED WITH COVID-19

COVID-19 TANISI ALAN ÇOCUKLARIN B LENFOSİT HÜCRELERİNİN VE ALT GRUPLARININ AKIŞ SİTOMETRİK DEĞERLENDİRİLMESİ

Mehmet Fatih ORHAN[1], Öner ÖZDEMİR[2], Gülay TRAK[3]

[1]Sakarya University, Faculty of Medicine, Department of Pediatric Hematology and Oncology, Sakarya, Turkiye
[2]Sakarya University, Faculty of Medicine, Department of Pediatric Immunology and Allergy Diseases, Sakarya, Turkiye
[3]Sakarya University, Faculty of Medicine, Department of Medical Microbiology, Sakarya, Turkiye

ORCID IDs of the authors: M.F.O. 0000-0001-8081-6760; Ö.Ö. 0000-0002-5338-9561; G.T. 0000-0002-4120-7249

Cite this article as: Orhan MF, Oner O, Trak G. A flow cytometric evaluation of B lymphocyte cells and subgroups of children diagnosed with COVID-19. J Ist Faculty Med 2022;85(3):291-5. doi: 10.26650/IUITFD.1079629

ABSTRACT

Objective: During viral infections, antibody production of B cells are critical for protective immunity. It is known that the COVID-19 disease has a milder course in children. It is crucial to evaluate the causes of this situation from a pediatrician's perspective to determine the treatment goals of the disease. We aimed to examine the flow cytometric changes in B cells and subtypes observed in children diagnosed with the COVID-19 infection.

Materials and Methods: This is a prospective cohort study including 22 children aged 0-18 who had been diagnosed with COVID-19. $CD19^+$B cells, $CD27^-IgD^+$ naive B, $CD21^{low}$ immature B, $CD21^{low}CD38^{low}$ active B, $CD27^-IgD^-$ double-negative B, $CD27^-$ non-memory B, $CD27^+$ memory B, $CD27^+IgD^-$ switched memory B, and $CD27^+IgD^+$ non-switched memory B cells were studied using flow cytometry.

Results: B cells counts decreased as a percentage in the 2-5 years age group and the 10-16 age group as an absolute number. Naive and non-memory B cell frequencies increased in the 5-10 years old and over 16 years old groups. Double negative B cells were normal in all age groups. Non-memory B cells increased in the 5-10 and over 16 years old groups, whereas memory B cells decreased. In all groups, switched memory B cells decreased. Non-switched memory B cell counts were within reference ranges in all groups except for the over 16 years group.

Conclusion: Although the decrease in B cell count is associated with the severity of the disease, naive B cell subgroups did not decrease in the pediatric patients included in the study. All groups showed increased switched memory B cell counts, in accordance with the literature. Unlike adults, naive B cells, non-switched memory B cells, and double-negative B cells were normal in children.

Keywords: COVID-19, child, immunology, flow cytometry, B-lymphocyte subgroups

ÖZET

Amaç: Viral enfeksiyonlar sırasında B hücrelerinin antikor üretimi, koruyucu bağışıklık için kritiktir. Çocuklarda COVID-19 hastalığının daha hafif seyrettiği bilinmektedir. Bu durumun nedenlerini çocuk doktoru gözüyle değerlendirmek, hastalığın tedavi hedeflerini belirlemek açısından çok önemlidir. COVID-19 enfeksiyonu tanısı alan çocuklarda gözlenen B hücre ve alt tiplerinde akım sitometrik değişiklikleri incelemeyi amaçladık.

Gereç ve Yöntem: Çalışmamız 0-18 yaş arası COVID-19 teşhisi konulan 22 çocuğu içeren prospektif kohort bir araştırmadır. $CD19^+$B hücreleri, $CD27^-IgD^+$ saf B, $CD21^{düşük}$ olgunlaşmamış B, $CD21^{düşük}CD38^{düşük}$ aktif B, $CD27^-IgD^-$ çift negatif B, $CD27^-$ bellek B, $CD27^+$ bellek B, $CD27^+IgD^-$ dönüşmüş (switched) bellek B, $CD27^+IgD^+$ dönüşmemiş (non-switched) bellek B hücreleri akış sitometrisi ile incelenmiştir.

Bulgular: B hücre sayısı 2-5 yaş grubunda yüzde olarak, 10-16 yaş grubunda ise mutlak sayı olarak azaldı. 5-10 yaş ve 16 yaş üstü gruplarda naif ve hafıza dışı B hücrelerinin oranları arttı. Çift negatif B hücreleri tüm yaş gruplarında normaldi. Bellek dışı B hücreleri 5-10 yaş arasında ve 16 yaş üzerinde artarken, aynı gruplarda bellek B hücreleri azaldı. Dönüşmüş bellek B hücreleri tüm yaş gruplarında azaldı. Dönüşmemiş bellek B hücreleri, 16 yaşın üzerinde azaldı ve diğer tüm yaş gruplarında normal görünüyordu.

Sonuç: B hücre sayısındaki azalma hastalığın şiddeti ile ilişkili olmasına rağmen, çalışmaya dâhil edilen çocuk hastalarımızda naif B hücre alt gruplarında azalma olmadı. Literatüre uygun olarak tüm gruplarda dönüşmüş bellek B hücreleri arttı. Çocuklarda yetişkinlerden farklı olarak naif B hücreleri, dönüşmemiş bellek B hücreleri ve çift negatif B hücreleri normaldi.

Anahtar Kelimeler: COVID-19, çocuk, immünoloji, akım sitometrisi, B-lenfosit alt grupları

Corresponding author/İletişim kurulacak yazar: Mehmet Fatih ORHAN – forhan@sakarya.edu.tr

Submitted/Başvuru: 26.02.2022 • **Revision Requested/Revizyon Talebi:** 07.03.2022 •
Last Revision Received/Son Revizyon: 13.05.2022 • **Accepted/Kabul:** 16.05.2022 • **Published Online/Online Yayın:** 16.06.2022

Content of this journal is licensed under a Creative Commons Attribution-NonCommercial 4.0 International License.

INTRODUCTION

Adaptive immunity is a part of the elaborate mosaic of the immune system which demonstrates complicated relationships and cooperation between T, B, and the subsets of these cells. During viral infection, B cells are critical for antibody production and protective immunity (1). It is known that the COVID-19 disease has a milder course in children. It is vital to increase our knowledge about how COVID-19 disease progresses in the different patient groups (2). We believe that evaluating the causes of this condition from a paediatrician's perspective will significantly contribute to the literature to determine the treatment goals of the disease (3-7). This study aimed to examine flow cytometric changes in B cells and subtypes during diagnosis in paediatric patients diagnosed with COVID-19 with SARS-CoV-2 PCR positivity.

MATERIALS AND METHODS

The study included 22 children aged 0-18 years who were positive for COVID-19 when they applied to the Pediatric Emergency Service and the COVID Outpatient Clinic. The patients were diagnosed as having COVID-19 using the SARS-CoV-2 PCR test from a oropharynx/nasopharynx swab sample. In addition, 4 ml blood samples were taken from the patients to K2 EDTA tube. The panel containing B cells and their subgroups was studied using flow cytometry. The patients were divided into four groups (2-5 years old, 5-10 years old, 10-16 years old, over 16 years old).

In the flow cytometry panel; $CD19^+$ B cells, $CD27^-IgD^+$ naive B, $CD21^{low}$ immature B, $CD21^{low}CD38^{low}$ active B, $CD27^-IgD^-$ double-negative B, $CD27^-$ non-memory B, $CD27^+$ memory B, $CD27^+IgD^-$ switched memory B (class-switched memory B), and $CD27^+IgD^+$ non-switched memory B were studied. The results obtained were evaluated according to the normal reference ranges obtained from studies in healthy children and adolescents (0-18 years) (8-11).

On the same day that the tests were to be performed, EDTA-K2 tubes were used to draw 4 ml blood samples which were immediately transferred to the microbiology laboratory. Monoclonal antibodies were applied to samples normalized to 1×10^6 cells per ml. Flow cytometry was utilized to analyse lymphocyte subsets with use of the FACSDiva software on a FACSCantoII device (Becton Dickinson, San Jose, CA, USA) (12). Anti-CD38 FITC, anti-CD21 PE, anti-CD27 PerCP-Cy5.5, anti-CD45 APC, anti-Human IgD PE-Cy and anti-CD19 APC-Cy7 were placed in the tubes. After incubation (room temperature, 20 minutes), erythrocyte elimination was performed with 2-3 ml of Lysing Solution (Becton Dickinson, San Jose, CA 95131 USA) under the same conditions. The cells were then washed with 2 mL Phosphate Buffered Saline (PBS) and suspended in a 500 µL solution (1% paraformaldehyde in PBS) and resultant samples were kept in the dark at 2-8°C immediately before analysis.

Children diagnosed with primary immunodeficiency disease, who were affected by steroid therapy, who received chemotherapy in the last 15 days, and whose parents did not allow the use of their child's data were not included in the study. This study was approved by the Clinical Research Ethical Committee of Sakarya University Faculty of Medicine (Date: 21.04.2021, No: 03).

Statistical analysis

Whether the numerical variables were normally distributed or not was analysed with the Kolmogorov-Smirnov test. Normally distributed numerical variables were expressed as mean ± standard deviation, non-normally distributed numerical variables were expressed as median (minimum-maximum), and categorical variables were expressed as numbers and percentages. When comparing the numerical variables of independent groups, the Student's t-test was used for those with normal distribution, and Mann Whitney U test was used for those without normal distribution. SPSS Statistics for Windows, Version 21.0. (IBM Corp. Armonk, NY: USA. Released 2012) package program was used for statistical analysis.

RESULTS

To compare the values obtained in this study with the normal, the children were divided into four age groups (2-5 years, 5-10 years, 10-16 years, and over 16 years old). Percentage and absolute counts of lymphocytes were higher than reference ranges above 16, while it was normal in other age groups (Figure 1). B cell percentage was decreased in the 2-5 age group and the 10-16 age group as an absolute number. The $CD19^+$B cell count was normal in the group above 16 years of age with high total and percentage lymphocyte counts (Table 1).

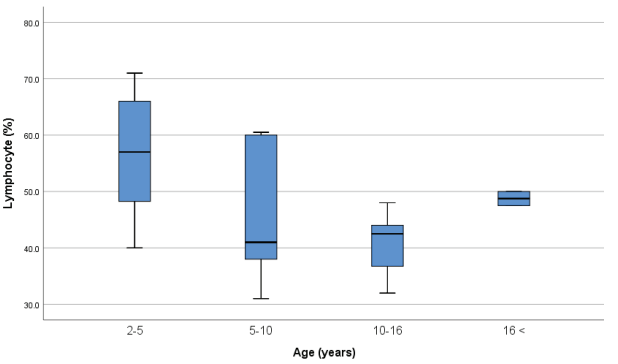

Figure 1: Lymphocyte percentage values by age groups

The percentages of naive and non-memory B cells increased in the 5-10 and over 16 year age groups. The percentage of immature B cells and activated B cells increased in the 10-16 age group (Table 2). Also, in the over

Table 1: Median (min-max) values of percentage and absolute numbers of lymphocytes and B cells by age groups in children with COVID-19

	2-5 Years (n=8)	5-10 Years (n=5)	10-16 Years (n=7)	>16 Years (n=2)
Lymphocyte (%)	57 (40-71)	41 (31-60)	43 (32-48)	49 ⇑ (48-50)
Lymphocyte (/µL)	4283 (1835-7205)	2874 (2128-4459)	2273 (1955-2938)	2538 ⇑ (1990-3085)
CD19⁺B Cell (%)	12 ⇓ (5-35)	12 (9-18)	9 (6-13)	16 (11-21)
CD19⁺B Cell (/µL)	444 (83-1702)	397 (251-520)	172 ⇓ (132-370)	427 (217-636)

⇑ increased, ⇓ decreased

Table 2: Median (min-max) values of percentage and absolute numbers of B cell subgroups by age group in children with COVID-19

	2-5 Years (n=8)	5-10 Years (n=5)	10-16 Years (n=7)	>16 Years (n=2)
Naive IgD⁺CD27⁻ B Cell (%)	86 (72-92)	83 ⇑ (54-93)	75 (26-82)	83 ⇑ (80-86)
Naive IgD⁺CD27⁻B Cell (/µL)	384 (60-1563)	350 (159-437)	129 (35-279)	348 (186-509)
Immature CD21^{low}B Cell (%)	10 (3-30)	6 (1-29)	14 ⇑ (5-23)	7 (3-11)
Immature CD21^{low}B Cell (/µL)	66 (21-128)	55 (12-86)	20 (17-53)	39 (7-71)
Activated CD21^{low}CD38^{low}B Cell (%)	5 (3-18)	5 (2-21)	11 ⇑ (3-19)	6 (2-11)
Activated CD21^{low}CD38^{low}B Cell (/µL)	31 (13-106)	13 (8-75)	17 (10-48)	36 ⇑ (5-67)
Double negative IgD⁻CD27⁻B Cell (%)	4 (0-8)	4 (2-17)	10 (4-56)	7 (1-14)
Double negative IgD⁻CD2^{7-} B Cell (/µL)	24 (4-77)	15 (8-49)	17 (6-76)	50 (30-70)
Non-memory CD27⁻ B Cell (%)	90 (78-95)	87 ⇑ (71-95)	82 (73-86)	95 ⇑ (91-100)
Non-memory CD27⁻ B Cell (/µL)	411 (64-1600)	366 (208-452)	145 (105-292)	398 (216-579)
Memory CD27⁺B Cell (%)	10 (1-22)	13 ⇓ (1-29)	17 (2-27)	1 ⇓ (1-1)
Memory CD27⁺ B Cell (/µL)	58 (18-111)	55 ⇓ (21-85)	35 ⇓ (22-78)	29 ⇓ (1-57)
Switched memory IgD⁻CD27⁺B Cell (%)	3 ⇓ (0-20)	1 ⇓ (0-2)	5 ⇓ (2-18)	4 ⇓ (1-8)
Switched memory IgD⁻CD27⁺B Cell (/µL)	13 ⇓ (0-75)	4 ⇓ (2-6)	7 ⇓ (5-48)	26 (1-51)
Non-switched memory IgD⁺CD27⁺B Cell (%)	6 (0-2)	12 (4-27)	11 (1-23)	0 ⇓ (0-1)
Non-switched memory IgD⁺CD27⁺B Cell (/µL)	35 (0-111)	50 (17-78)	15 ⇓ (3-70)	3 ⇓ (0-6)

⇑ increased, ⇓ decreased

16 years age group, activated B cells increased in terms of absolute count.

Double negative B cells were normal in all age groups. Non-memory B cells increased over the age of 5-10 and over 16, whereas memory B cell counts decreased in these groups (Table 2).

All age groups demonstrated decreased switched memory B cell counts. Non-switched memory B cell and marginal zone like B cell counts decreased over the age of 16, while counts were normal in all other age groups (Table 2).

DISCUSSION

It has been found that patients with severe COVID-19 show an overall decrease in lymphocyte count and decreased B cells (13,14). B cells and NK cells in particular are also reduced in this disease (15). A study of 40 children who developed pneumonia due to COVID-19 showed lower percentages of CD19+B lymphocytes than RSV pneumonia (16). Within the scope of our research, a decrease was found in the 2-5 years age group as a percentage and the 10-16 years age group as the absolute number. However, none of our patients progressed severely enough to go to intensive care. It was evaluated that looking at pure B cell numbers cannot be a function parameter in predicting prognosis, and B cell subgroups should also be examined.

Interestingly, the majority of CD20-depleted individuals infected with SARS-CoV-2 with autoimmunity are known to recover (17, 18). This reveals the importance of B lymphocytes in the pathogenesis of COVID-19 disease. If the immunopathology of this disease can be explained, immunotherapy will also be possible (19).

It has been reported that the percentages of B cells increase in patients with severe disease, but the absolute number of B cells gradually decreases (20). Our study found that although the lymphocyte count increased in the 16-year-old group closest to adult age, B cells did not increase.

No statistically significant change in disease severity was reported in the B cell subgroups in one study, although there were different trends when all patients were considered together (21, 22). In another study, $CD19^+$B cells, like other lymphocyte subgroups, were lower than the standard limit one week after the onset of the disease, and this nadir was found in the second week. As of the third week, this number gradually increases and continues until the 5^{th} week, but this value cannot reach a healthy standard value in 5^{th} week. In general, B cell count decline has been associated disease severity (23). In a study in which 60 patients with COVID-19 were evaluated before and after treatment, the decrease in B cells and the total lymphocyte count was associated with the severity of the disease. Compared with healthy controls, this study showed that the absolute lymphocyte and B cell counts decreased significantly. Thirty-seven patients who showed a clinical response demonstrated increased B cells following therapy. A low B cell count that persists after treatment has been associated with a poor prognosis (24). Considering that our patients' tests were ordered at diagnosis–not after treatment, it is impossible to speculate on this issue. However, children in the youngest age group 2-5 years and 5-10 years started with a low B cell count, did not develop any intensive care needs after one week of follow-up and were discharged.

In another study, while plasma cells increased significantly among B cells, naive B cell measurements showed a decline (25). The percentages of naive B cell subtypes, switched memory ($CD27^+IgD^-$) and non-switched ($CD27^+IgD^+$) memory B cells, are significantly reduced in COVID-19 patients. In contrast, the frequencies of double-negative ($CD27^-IgD^-$) B cells and $CD27^+$-CD38+ generally increase enormously (12). Naive B cell subgroups did not decrease in our paediatric patients included in the study, while increases were observed in switched memory B cells, similar to the literature. Non-switched memory B cells showed a decline in only the over 16 age group. The double negative B cell group also did not decrease and remained normal. These results showed us that, unlike adults, our patients had normal levels of naive B cells, non-switched memory B cells and double-negative B cells (Table 2).

In another study, the percentages of memory B ($CD27^+CD19^+$) cells were found to be similar in the groups (20). In our research, it was observed that memory B cells generally decreased. There was a decrease in absolute numbers and percentages of memory B cells in the 5-10, 10-15, and over 16 years age groups. The change in memory B cells was expected in the 2-5 age group, due their recent exposure to multiple vaccines.

As a result, children constituted the least damaged group during this global pandemic, and studies continue to vaccinate this group. Therefore, it is essential to reveal what is different in children. As a result of this study, different from adults in children, naive IgD^+CD27^-B cell, double negative IgD^-CD27^-B cell and non-switched memory/marginal zone like IgD^+CD27^+B cells did not decrease but remained normal. This outbreak has once again highlighted the shortcomings in our ability to respond to and reduce new pathogen outbreaks.

A limited number of patients were included because of budgetary constraints and only B cell profiles at the time of diagnosis could be studied. If it had been possible to enrol more patients, particularly into the over 16 years age group, the difference between this age group (near adult age) and other childhood periods may have been better demonstrated.

Ethics Committee Approval: This study was approved by the Clinical Research Ethical Committee of Sakarya University Faculty of Medicine (Date: 21.04.2021, No: 03).

Peer Review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study- M.F.O., Ö.Ö.; Data Acquisition- M.F.O., G.T.; Data Analysis/Interpretation- M.F.O., Ö.Ö.; Drafting Manuscript- M.F.O.; Critical Revision of Manuscript- M.F.O., Ö.Ö., G.T.; Final Approval and Accountability- M.F.O., Ö.Ö., G.T.; Material or Technical Support- G.T.; Supervision- Ö.Ö., M.F.Ö.

Conflict of Interest: The authors have no conflict of interest to declare.

Financial Disclosure: The authors declared that this study has received no financial support.

REFERENCES

1. Baker D, Roberts CA, Pryce G, Kang AS, Marta M, Reyes S, et al. COVID-19 vaccine-readiness for anti-CD20-depleting therapy in autoimmune diseases. Clin Exp Immunol 2020;202(2):149-61. [CrossRef]
2. Chiappelli F. COVID-19 Immunopathology & Immunotherapy. Bioinformation 2020;16(3):219-22. [CrossRef]
3. Comans-Bitter WM, De Groot R, Van den Beemd R, Neijens HJ, Hop WCJ, Groeneveld K, et al. Immunophenotyping of blood lymphocytes in childhood: Reference values for lymphocyte subpopulations. J Pediatr 1997;130(3):388-93. [CrossRef]
4. Deng Z, Zhang M, Zhu T, Zhili N, Liu Z, Xiang R, et al. Dynamic changes in peripheral blood lymphocyte subsets in adult patients with COVID-19. Int J Infect Dis 2020;98:353-8. [CrossRef]
5. Devogelaere J, D'hooghe MB, Vanderhauwaert F, D'haeseleer M. Coronavirus disease 2019: favorable outcome in an immunosuppressed patient with multiple sclerosis. Neurol Sci 2020;41(8):1981-3. [CrossRef]
6. He R, Lu Z, Zhang L, Fan T, Xiong R, Shen X, et al. The clinical course and its correlated immune status in COVID-19 pneumonia. J Clin Virol 2020;127:104361. [CrossRef]
7. Huang W, Berube J, McNamara M, Saksena S, Hartman M, Arshad T, et al. Lymphocyte Subset Counts in COVID-19 Patients: A Meta-Analysis. Cytom Part A 2020;97(8):772-6. [CrossRef]
8. Kebudi R, Kurucu N, Tuğcu D, Hacısalihoğlu Ş, Fışgın T, Ocak S, et al. COVID-19 infection in children with cancer and stem cell transplant recipients in Turkey: A nationwide study. Pediatr Blood Cancer 2021;68(6):e28915. [CrossRef]
9. Li H, Chen K, Liu M, Xu H, Xu Q. The profile of peripheral blood lymphocyte subsets and serum cytokines in children with 2019 novel coronavirus pneumonia. J Infect 2020;81(1):115-20. [CrossRef]
10. Liu J, Li S, Liu J, Liang B, Wang X, Wang H, et al. Longitudinal characteristics of lymphocyte responses and cytokine profiles in the peripheral blood of SARS-CoV-2 infected patients. EBioMedicine 2020;55:1027633. [CrossRef]
11. Machura E, Mazur B, Pieniążek W, Karczewska K. Expression of naive/memory (CD45RA/CD45RO) markers by peripheral blood CD4 + and CD8 + T cells in children with asthma. Arch Immunol Ther Exp (Warsz) 2008;56(1):55-62. [CrossRef]
12. Mathew D, Giles JR, Baxter AE, Oldridge DA, Greenplate AR, Wu JE, et al. Deep immune profiling of COVID-19 patients reveals distinct immunotypes with therapeutic implications. Science 2020;369(6508):eabc8511. [CrossRef]
13. Melek Arsoy HE, Özdemir Ö. Mysterious Side of COVID-19 Pandemic: Children. Istanbul Med J 2020;21(4):242-57. [CrossRef]
14. Nielsen S, Fang Y, Jackson K, Hoh R, Röltgen K, Stevens B, et al. Human B cell clonal expansion and convergent antibody responses to SARS-CoV-2. Cell Host Microbe 2020;28(4):516-525.e5. [CrossRef]
15. Orhan MF, Büyükavcı M. COVID-19'un tanı ve tedavi sürecinde hematolojik parametreler. J Biotechnol Strateg Heal Res 2020;4:123-7. [CrossRef]
16. Özdemir Ö. Coronavirus Disease 2019 (COVID-19): Diagnosis and Management (narrative review). Erciyes Med J 2020;42(3):242-7. [CrossRef]
17. Özdemir Ö. İmmün sistemin COVID-19 hastalığındaki rolü: Patogenezden tedaviye. Bostancı İ, editör. Çocuk Sağlığında SARSCoV- 2 (COVID-19). 1. Baskı. Ankara: Türkiye Klinikleri; 2020. p.14-21.
18. Özdemir Ö, Erkun O. Solving puzzle of the immunopathogenesis for management of COVID-19 disease. MOJ Immunol 2020;7(1):13-5.
19. Schatorjé EJH, Gemen EFA, Driessen GJA, Leuvenink J, van Hout RWNM, et al. Age-matched reference values for B-lymphocyte subpopulations and CVID classifications in children. Scand J Immunol 2011;74(5):502-10. [CrossRef]
20. Schatorjé EJH, Gemen EFA, Driessen GJA, Leuvenink J, van Hout RWNM, de Vries E. Paediatric reference values for the peripheral T cell compartment. Scand J Immunol 2012;75(4):436-44. [CrossRef]
21. Shearer WT, Rosenblatt HM, Gelman RS, Oyomopito R, Plaeger S, Stiehm ER, et al. Lymphocyte subsets in healthy children from birth through 18 years of age: The Pediatric AIDS Clinical Trials Group P1009 study. J Allergy Clin Immunol 2003;112(5):973-80. [CrossRef]
22. Tan M, Liu Y, Zhou R, Deng X, Li F, Liang K, et al. Immunopathological characteristics of coronavirus disease 2019 cases in Guangzhou, China. Immunology 2020;160(3):261-8. [CrossRef]
23. Wang F, Hou H, Luo Y, Tang G, Wu S, Huang M, et al. The laboratory tests and host immunity of COVID-19 patients with different severity of illness. JCI Insight 2020;5(10):e137799. [CrossRef]
24. Wang F, Nie J, Wang H, Zhao Q, Xiong Y, Deng L, et al. Characteristics of peripheral lymphocyte subset alteration in COVID-19 Pneumonia. J Infect Dis 2020;221(11):11762-9. [CrossRef]
25. Wen W, Su W, Tang H, Le W, Zhang X, Zheng Y, et al. Immune cell profiling of COVID-19 patients in the recovery stage by single-cell sequencing. Cell Discov 2020;6(1):31. [CrossRef]