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Lymphocyte Subsets in Mild COVID-19 Pediatric Patients

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What is already known about this topic?

- *Children experience mild COVID-19 disease. However, the reason for this phenomenon is not exactly apparent. Studies on non-immune and immune mechanisms are ongoing. It is now known that there is a negative correlation between disease severity and lymphocyte count, lymphocyte subsets, and particularly CD8+ T cell count in adult patients.*

What this study adds on this topic?

- *Lymphocyte count in pediatric COVID-19 patients with asymptomatic or mild disease is similar to that of healthy children. However, natural killer cells, T cell, and CD4+ T cell counts are increased.*

ABSTRACT

Objective: The reasons for a high prevalence of asymptomatic or mild coronavirus disease (COVID-19) and rare severe disease in children have been explained by non-immune and immune mechanisms. This study aimed to evaluate the immune system's response to severe acute respiratory syndrome coronavirus 2 by investigating lymphocyte subsets.

Materials and Methods: This study included 33 coronavirus disease positive children, of whom 12 had mild disease and 21 had an asymptomatic infection as the patient group and 26 age- and gender-matched healthy children as the control group. The demographic information, symptoms, physical examination findings, complete blood count, C-reactive protein (CRP), procalcitonin, and lymphocyte subsets were recorded in all subjects.

Results: Leukocyte, lymphocyte, monocyte count, and hemoglobin levels of our pediatric coronavirus disease patients were similar to the control group. Neutrophil was lower in the coronavirus disease cases compared to the control group. CRP and procalcitonin levels of asymptomatic cases were similar to the control group. B cell count, CD8+ T cell count, and CD4/CD8 ratio (dividing the CD4 cell count by the CD8 cell count) ratio were similar in the patient and control groups. Natural killer, T cell, and CD4+ T cell counts were significantly higher in the whole patient group compared to the control group.

Conclusion: One reason for mild severe acute respiratory syndrome coronavirus 2 infection in children may be an increase in some lymphocyte subsets such as natural killer cells, T cell, and CD4+ T cell. Understanding the answer to the question of why children develop more protective immunity to the virus could be an essential step for developing new treatments.

Keywords: SARS-CoV-2, COVID-19, lymphocyte subsets, children.

INTRODUCTION

In the final days of 2019, some pneumonia cases of unknown etiology were identified in Wuhan City, China. The causative agent was severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a member of the coronavirus family. The disease was then named by World Health Organization (WHO) the coronavirus disease 2019 (COVID-19).[1] Upon a rapid rise of COVID-19 cases worldwide, WHO declared a pandemic on March 11, 2020.[2] The virus' fast transmission, its high virulence, and severe course, especially in adults, have caused millions of people to be hospitalized and die worldwide.

Coronaviruses are enveloped, positive-sense, single-stranded RNA viruses. They are divided into 4 genera by their α, β, γ, and δ genomic structures. They can infect a wide variety of host species. α and β genera only infect mammals. Some human coronaviruses are responsible

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for upper respiratory tract infections. SARS-CoV-2 is a novel human coronavirus that belongs to the β coronavirus family.[3]

It is usually transmitted with respiratory droplets and via close contact with infected persons. Crowded places are the ideal environment for the spread of the disease. Given the number of children in the general population and the risk of exposure, children are an important source of the virus.[4] The virus enters the human body through respiratory mucosa and conjunctiva. The upper respiratory tract mucosa is the first viral replication site. The virus enters the cell using the angiotensin-converting enzyme 2 (ACE2) receptor, a functional receptor that is mainly expressed in the upper airways, lungs, heart, kidneys, intestines, and vascular endothelial cells.[5]

When viruses bind to host receptors (attachment), they enter host cells via endocytosis or membrane fusion (penetration). When the viral contents are released inside the host cells, the viral RNA enters the nucleus for replication. Viral mRNA is used to make viral proteins (biosynthesis). New viral particles are made (matured) and then released. Epithelial cells, alveolar macrophages, and dendritic cells are the 3 main components of innate immunity in the respiratory tract. Dendritic cells' and macrophages' duty as innate immune cells are to fight viruses until adaptive immunity is developed. T-cell responses are initiated by antigen presentation by dendritic cells and macrophages. These antigen-presenting cells move to the draining lymph nodes to present viral antigens to T cells. T helper and T cytotoxic cells play a pivotal role. T helper cells activate B cells to promote the production of virus-specific antibodies, while T cytotoxic cells kill virally infected cells.[3]

Although the SARS-CoV-2 virus mainly affects the respiratory system, it can also affect other organ systems. It may cause various clinical manifestations in adults, including an asymptomatic disease, mild upper respiratory tract infection, mild-to-severe pulmonary infection, and severe systemic inflammation characterized by acute respiratory distress syndrome and coagulation abnormalities. It is believed that viral load is taken, viral cell entry, the protective immune response, and the effects of an abnormally severe immune response, including the cytokine storm, play a role in the COVID-19's presentation.[5-6] Asymptomatic or mild disease in children causes the disease to become undiagnosed. Symptoms generally include fever, cough, sore throat, runny nose, myalgia, malaise, vomiting, diarrhea, and abdominal pain. The percentage of children requiring intensive care due to pneumonia is low. Severe disease has been reported in 1-5% of affected children. Death is extremely rare. The risk of severe disease is higher, especially in infants and patients with comorbidities. Multisystem inflammatory syndrome, a condition resembling the Kawasaki syndrome, which develops after acute infection and may have a severe presentation, has been defined in an increasing number of children.[5-7]

It is not entirely clear why children develop mild COVID-19 disease. It is believed that since children are more frequently exposed to seasonal coronaviruses and experience a greater number of viral infections and since some childhood vaccines keep their immune systems more active, they develop a more controlled and appropriate immune response against the virus. In addition, the expression of ACE2 receptors in the nasal epithelium and lower respiratory tract in children is different from that in adults.[2,4,8]

It is known that lymphopenia, and especially a decrease in T lymphocyte count in adults, is negatively associated with prognosis during COVID-19 disease.[1] Lymphocyte and its subsets may have an impact on the development of the protective immune system in children during COVID 19 disease. Herein, we discuss the numerical change of lymphocyte subsets and their effect on mild COVID-19 infection in children.

MATERIALS AND METHODS

Ethical Approval and Consent to Participate

This study was approved by Kayseri City Hospital clinical research ethics committee (April/2020; no.64). The study was conducted in accordance with the declaration of Helsinki. Informed consent was obtained from the parents of all patients and also from the adolescent patients themselves.

Subjects

This study enrolled a total of 33 pediatric patients who were hospitalized with COVID-19 at Kayseri City Hospital Pediatric Clinic between April 2020 and June 2020.

Inclusion and Exclusion Criteria

The inclusion criteria were as follows: detection of SARS-CoV-2 nucleic acids using real-time fluorescent polymerase chain reaction (PCR) in the throat and nasal swab sample in a pediatric patient with suspected COVID 19.

The exclusion criteria were as follows: having a neurological, allergic, immunological, cardiac, or other chronic disease and using medication due to chronic disease.

Clinical Classification of Disease Severity

The patients were either symptomatic or asymptomatic, and each patient had a history of contact with a COVID-19-positive person. Cases with positive COVID-19 PCR tests in the throat and nasal swab samples were accepted as COVID 19.

In Turkey, at the beginning of the pandemic, pediatric cases with the asymptomatic or mild disease had been hospitalized for contact isolation. Therefore, all of our patients were hospitalized. Symptomatic patients were administered azithromycin (10 mg/kg/day, first day; then, 5 mg/kg/day for 4 days) per oral route. The control group was composed of age- and gender-similar healthy children.

COVID 19 patients were classified as a follow-up. Asymptomatic: cases with no clinical symptoms and signs and normal chest imaging. Mild: patients with signs of acute upper respiratory tract infection (fever, fatigue, myalgia, cough, sore throat, runny nose, and sneezing) or digestive system symptoms (nausea, vomiting, abdominal pain, and diarrhea). Moderate: pneumonia without significant hypoxemia (frequent fever and cough) and chest computed tomography with lesions.[3]

General Laboratory Tests

The demographic information, symptoms, physical examination findings, C-reactive protein (CRP), procalcitonin, complete blood count, and lymphocyte subsets were recorded in all subjects.

Full blood count was performed with an automatic hematological analyzer.

Flow Cytometry Assay

Peripheral blood lymphocyte subsets were determined using flow cytometry. EDTA (Ethylene diamine tetra acetic acid)-anticoagulated peripheral blood (2 mL) was collected from patients with COVID-19 before initial treatment. All samples were tested within 6 hours of being obtained. CD3+/CD4+/CD8+ T-cell, CD19+ B-cell, and CD16+CD56+ natural killer (NK)-cell counts (cells/μL) were measured by multiple-color flow cytometry with a human monoclonal anti-CD3-fluorescein isothiocyanate, anti-CD4-phycoerythrin cyanine7, anti- CD8-allophycocyanin H7, anti-CD19-APC, and anti-CD16-56PE antibodies according to the manufacturer's instructions. The cells were analyzed and calculated using the clinical program of the FACS Lyric flow cytometer device.

Statistical Analysis

Study data were analyzed with The Statistical Package for Social Sciences version 25.0 software (IBM Corp.; Armonk, NY, USA). Descriptive statistics were presented as number (n), percentage (%), mean ± standard deviation ((x)' ± SD), median (M), and interquartile range. Normality of the distribution of numerical variables was tested with the Shapiro–Wilk normality test and Q–Q graphics. Mann–Whitney *U* test was used to compare non-normally distributed variables between the 2 groups. One-way analysis of variance (ANOVA) was used to compare

Table 1. The Demographic Characteristics, Complete Blood Count, CRP, Procalcitonin, and Lymphocyte Subsets of All, Symptomatic, and Asymptomatic Patients as well as the Control Group Were Shown

Characteristics	All COVID-19 Cases (n = 33)	Symptomatic COVID-19 Patients (Mild Disease) (n = 12)	Asymptomatic COVID-19 Cases (n = 21)	Healthy Control (n = 26)
	114.7 ± 61.3	117.3 ± 59.9	113.3 ± 63.5	133.2 ± 67.4
¥Gender (male/female), N (%)	(16/17) (48.5/51.5)	(4/8) (33.3/66.7)	(12/9) (57.1/42.9)	(15/11) (57.7/42.3)
Blood count, $\times 10^9$/L				
‡Leucocytes Median (25-75p)	6.220 (5.195-7.390)	5.685 (4.682-6.882)	6.580 (5.895-8.005)	7.095 (5.772-8.282)
†Hemoglobin (g/dL)	13.2 ± 1.4	13.1 ± 0.7	13.2 ± 1.7	13.3 ± 1.4
†Hemotocrit (%)	38.5 ± 4.0	38.5 ± 2.6	38.6 ± 4.7	39.3 ± 3.4
†Mean corpuscular volume (fL)	77.9 ±4.3	77.1 ± 3.8	78.4 ± 4.7	80.1 ± 3.8
†Red blood cell distribution width (%)	12.7 ± 0.9	12.9 ± 0.6	12.6 ± 1.0	12.7 ± 0.9
‡Platelet	274 (228-310)	240 (200.5-293.5)* $P = .015$	279 (266.5-316)	298.5 (262.5-354)
ᵍC-reactive protein (mg/L)	1.40 (0.60-3.75)	2.50 (1.12-5.92)	1.40 (0.50-3.25)	
ᵍProcalcitonin (ng/dL)	0.05 ± 0.03	0.06 ± 0.04	0.04 ± 0.01	
Flow cytometry, $\times 10^6$/L				
ᵍNeutrophil	2975 (2232-3558)* $P = .001$	2438 (1958-3501)* $P = .004$	3069 (2481-3621)* $P = .036$	3971 (3396-4793)
‡Monocyte	506 (374-715)	567 (311-779)	485 (402-693)	574 (446-663)
‡Lymphocyte	2301 (1915-3177)	1996 (1630-2794)	2380 (2047-3551)	2091 (1787-2521)
‡NK cell	252 (147-327)* $P = .035$	202 (119-289)	282 (172-332)* $P = .028$	199 (100-266)
‡B cell	307 (206-580)	242 (184-441)	414 (214-664)	363 (282-521)
‡ T cell	1818 (1397-2098)* $P = .029$	1620 (1256-2076)	1838 (1433-2182)	1489 (1108-1778)
‡CD4 cell	923 (826-1288)* $P = .027$	960 (827-1255)	923 (824-1337)	841 (643-992)
‡CD8 cell	711 (483-951)	483 (428-839)	797 (559-984)	580 (405-758)
†CD4/CD8 ratio	1.70 ± 0.78	1.76 ± 0.61	1.66 ± 0.87	1.61 ± 0.82

*Significant difference compared to control group, $P < .05$.
Data are expressed as mean ± standard deviation, median (1st quarter/3rd quarter) and n (%).
†One-way analysis of variance, ¥Pearson chi-square test with exact method, ‡Kruskal–Wallis analysis, ᵍMann–Whitney *U* test
COVID-19, coronavirus disease; NK, natural killer cell; CRP, C-reactive protein.

normally distributed variables between 3 groups. Tukey honestly significant difference was used as the multiple comparison test for ANOVA. Kruskal–Wallis test was used to compare non-normally distributed variables between 3 groups. If the Kruskal–Wallis test indicated significant inter-group differences, the Dunn–Bonferroni test was used as the multiple comparison test. Categoric variables were compared by Pearson's chi-square test. If the latter indicated a significant difference, inter-group differences were sought with the two proportion Z test with Bonferroni correction. A *P*-value of less than .05 was considered statistically significant.

RESULTS

Clinical Characteristics of Patients with COVID-19

In total, 33 children with COVID-19 (12 symptomatic cases and 21 asymptomatic cases) and 26 healthy children enrolled as the control group had similar age and gender distribution. The symptomatic patients (n = 12) had symptoms of an acute upper respiratory tract infection, including fever, cough, myalgia, malaise, vomiting, and diarrhea.

Physical examination of the symptomatic patients revealed findings consistent with an upper respiratory tract infection. No patient had pneumonia. All symptomatic patients had mild disease. Twenty-one asymptomatic patients had a normal physical examination. All patients were discharged after 5 days with full recovery.

Complete Blood Count, Acute Phase Reactants, and Lymphocyte Subsets

The demographic characteristics, complete blood count, CRP, procalcitonin, and lymphocyte subsets of all symptomatic and asymptomatic patients and the control group were shown in Table 1. There was no significant difference between the groups with respect to leukocyte count and hemoglobin value. Platelet count was significantly lower in the symptomatic group compared to the control group. CRP and procalcitonin were not elevated in the patient groups. Neutrophil count was considerably lower in each patient group compared to the control group. Monocyte, lymphocyte, B cell, CD8+ T cell counts, and CD4/CD8 ratio were similar in the patient and control groups. NK cell, T cell, and CD4+ T cell counts were significantly higher in all patients compared to the control group.

DISCUSSION

Our study revealed that the whole pediatric patient group that was composed of asymptomatic and mild cases had a lower neutrophil count; higher NK cell, T cell, and CD4+ T cell counts; and similar leukocyte, lymphocyte, monocyte, CD8+ T cell counts, and CD4/CD8 ratio compared to the controls.

Lymphocytes have an essential role in defense against viruses. CD4+ T lymphocytes produce potent cytokines to further activate the immune system and help B lymphocytes produce antibodies. CD8+ T lymphocytes destroy virus-infected cells to reduce viral load and limit the viral spread.[8]

In adults, SARS-CoV-2 infection causes lymphopenia, depending on disease severity. Lymphopenia appears to be related to apoptosis and cell death during cytokine release. Studies on adults have reported that a more significant drop occurs in CD8+ T lymphocyte count. In contrast to adults with severe disease, children with mild disease have similar or increased T lymphocyte counts.[8,9]

Studies on COVID-19 and lymphocyte subsets in adults have indicated that they show a negative correlation, especially with disease severity and outcome. Deng Z et al[1] found significantly lower CD3+, CD4+, and CD8+ T cell counts in patients with severe COVID-19 disease compared to patients without severe COVID-19 disease. The authors suggested that these findings were related to disease severity, progression, and prognosis. Chen J et al[6] found a negative correlation between disease severity and CD3+, CD4 +, and CD8+ T lymphocyte counts. They interpreted that these findings may indicate that symptomatic patients experience some immunological disorders. Kazancıoğlu S et al[10] reported an increased granulocyte count and reduced lymphocyte, CD3+ T cell, CD4+ T cell, NK cell, and monocyte counts in patients with severe COVID-19 disease. Gan J et al[11] reported that the number of lymphocyte subsets was correlated to a favorable outcome in patients with COVID-19 pneumonia. Qin et al[12] reported increased levels of inflammatory cytokines, a higher leukocyte count, and lower lymphocyte and T cell counts in patients with severe infection. Jiang et al[13] reported that patients with COVID-19 had severely depleted CD3+ T, CD4+ T, CD8+ T cell counts, with the depletion in CD8+ T cells being more severe. In a systematic review, Li et al[14] reported that COVID-19 progression and mortality showed a significant negative correlation with lymphocyte count but not CD3+, CD4+, CD8+ T cell, B cell, and NK cell counts. Sun et al[15] found that CD8+ T cell count was lower in severe and critical diseases. They interpreted this finding as being an independent predictor of disease severity. Wang et al[16] reported that patients with severe COVID-19 had more severely reduced CD4+ T cell, CD8+ T cell, B cell, and NK cell counts. They also reported that CD8+ T cells tended to be an independent predictor of disease severity and treatment efficacy. Lymphopenia and hypercoagulopathy are now considered the signs of a poor prognosis in adult patients.[17]

Children experience an asymptomatic or mild disease characterized by fever, cough, and gastrointestinal symptoms.[18] Among inflammatory markers, CRP and procalcitonin are normal in a majority of patients. A procalcitonin level above 0.5 ng/mL indicates a bacterial co-infection.[19] Hepatic enzymes, muscle enzymes, and D-dimer may increase in severe or critical cases. Anemia and abnormal platelet count are rare. White blood cell count is normal in most cases. Leukopenia is the most common white blood cell abnormality. Our patients had a lower neutrophil count than healthy children. While platelet count was lower in our symptomatic patients, CRP and procalcitonin were similar to the control group.

Lymphopenia is rare in children than adults.[17,20] In a study, lymphopenia was found in only 3.5% of 71 pediatric COVID-19 cases.[21] In a review article, Henry BM et al[22] reported that, unlike adults, pediatric COVID-19 cases usually have inconsistent changes in leukocyte indices, suggesting that these parameters do not appear as reliable markers of disease severity. Li et al[23] categorized 125 pediatric COVID-19 cases as

either upper respiratory tract infection or pneumonia and found no significant difference between CD4+ T cell, CD8+ T cell, B cell, and NK cell counts and CD4+/CD8+ cell ratio. However, the percentage of regulatory (CD4+ CD25+) T cells was lower in the pneumonia cases. Li et al[24] compared 40 pediatric cases of COVID-19 pneumonia and 16 pediatric cases of respiratory syncytial virus pneumonia and reported a higher CD8+ T cell count in COVID-19 (+) patients. They suggested that an effective CD8+response may be related to mild to moderate symptoms in children with COVID-19 pneumonia. Lu et al[25] reported that a decrease in the initial number of T cells, T helper cells, and T cytotoxic cells is a valuable indicator for the severity of the disease in children with SARS-CoV-2 infection. They emphasized that the severe decrease in the number of T cells and T helper cells in pediatric patients with critical SARS-CoV-2 infection may be closely related to the cytokine storm caused by immune dysregulation.

There was no decrease in lymphocyte count in our COVID-19 pediatric patients. Moreover, some lymphocyte subsets such as NK cell, T cell, and CD4+ T cell counts were increased. This may be due to the fact that the majority of our patients were asymptomatic, and the remainders suffered a mild disease. Children may be developing a more protective immune response to the virus than adults. These limits viral spread in the body and prevents an excessive immune response from being developed, leading to absent or limited systemic inflammation.

CONCLUSION

Asymptomatic and mild COVID 19 pediatric patients had lower neutrophil counts, similar lymphocyte counts, and higher NK cell, T cell, and CD4 cell counts compared to the healthy children. One reason for mild SARS-CoV-2 infection in children may be an increase in some lymphocyte subsets such as NK cell, T cell, and CD4 cell. The absence of lymphopenia and no decrease in lymphocyte subsets in pediatric patients with COVID 19 seem to be related to mild illness. Understanding why children develop protective immunity to the virus and adults develop an extreme immune response could be an important step toward developing new treatments.

The limitations of our study include a small patient number and the lack of analysis of the change in study parameters with treatment. It is not possible to evaluate the immune system only with the lymphocyte subset. The absence of a serious disease group in the study is another limitation. An additional limitation is that the cytokine level has not been studied.

Ethics Committee Approval: This study was approved by Ethics committee of Kayseri City Hospital (Approval No: April/2020; no.64).

Informed Consent: Written informed consent was obtained from the patients who agreed to take part in the study.

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REFERENCES

1. Deng Z, Zhang M, Zhu T, et al. Dynamic changes in peripheral blood lymphocyte subsets in adult patients with COVID-19. *Int J Infect Dis*. 2020;98:353-358. [CrossRef]
2. Shrestha R, Shrestha L. Coronavirus disease 2019 (Covid-19): a pediatric perspective. *JNMA J Nepal Med Assoc*. 2020;58(227):525-532. [CrossRef]
3. Üzel VH, Yılmaz K, Şen V, et al. Evaluation of hematological parameters of children diagnosed with COVID-19: single-center experience. *Turk Arch Pediatr*. 2021;56(5):463-468.
4. Çokuğraş H, Önal P. SARS-CoV-2 infection in children. *Turk Pediatri Ars*. 2020;55(2):95-102. [CrossRef]
5. Hendricks CL, Green RJ. COVID-19 in children: should we be Worried? *S Afr Med J*. 2020;110(9):864-868. [CrossRef]
6. Chen J, Han T, Huang M, et al. Clinical characteristics of asymptomatic carriers of novel coronavirus disease 2019: a multi-center study in Jiangsu Province. *Virulence*. 2020;11(1):1557-1568. [CrossRef]
7. Munro APS, Faust SN. COVID-19 in children: current evidence and key questions. *Curr Opin Infect Dis*. 2020;33(6):540-547. [CrossRef]
8. Suratannon N, Dik WA, Chatchatee P. Hagen PM van. COVID-19 in children: heterogeneity within the disease and hypothetical pathogenesis. *Asian Pac J Allergy Immunol*. 2020;38:170-177.
9. Cavallo F, Rossi N, Chiarelli F. Novel coronavirus infection and children. *Acta Biomed*. 2020;91(2):172-176. [CrossRef]
10. Kazancioglu S, Yilmaz FM, Bastug A, et al. Lymphocyte subset alteration and monocyte. *Viral Immunol*. 2020;00:1-10.
11. Gan J, Li J, Li S, Yang C. Leucocyte subsets effectively predict the clinical outcome of patients With COVID-19 pneumonia: a retrospective case-control study. *Front Public Health*. 2020;8:299. [CrossRef]
12. Qin C, Zhou L, Hu Z, et al. Dysregulation of immune response in patients with COVID-19 in Wuhan, China. *SSRN Journal*. 2020;53:1689-1699. [CrossRef]
13. Jiang M, Guo Y, Luo Q, et al. T cell subset counts in peripheral blood can be used as discriminatory biomarkers for diagnosis and severity prediction of COVID-19. *J Infect Dis*. 2020;222(2):198-202. [CrossRef]
14. Lu Q, Wang Z, Yin Y, Zhao Y, Tao P, Zhong P. Association of peripheral lymphocyte and the subset levels With the progression and mortality of COVID-19: a systematic review and meta-analysis. *Front Med (Lausanne)*. 2020;7:558545. [CrossRef]
15. Sun Y, Dong Y, Wang L, et al. Characteristics and prognostic factors of disease severity in patients with COVID-19: the Beijing experience. *J Autoimmun*. 2020;112:102473. [CrossRef]
16. Wang F, Nie J, Wang H, et al. Characteristics of peripheral lymphocyte subset alteration in COVID-19 pneumonia. *J Infect Dis*. 2020;221(11):1762-1769. [CrossRef]
17. Kosmeri C, Koumpis E, Tsabouri S, Siomou E, Makis A. Hematological manifestations of SARS-CoV-2 in children. *Pediatr Blood Cancer*. 2020;67(12):e28745. [CrossRef]
18. Martins MM, Prata-Barbosa A, Magalhães-Barbosa MC de, Cunha AJLAD. Clinical and laboratory characteristics of Sars-Cov-2 infection in children and adolescents. *Rev Paul Pediatr*. 2020;39:e2020231. [CrossRef]

19. Chen ZM, Fu JF, Shu Q, et al. Diagnosis and treatment recommendations for pediatric respiratory infection caused by the 2019 novel coronavirus. *World J Pediatr*. 2020;16(3):240-246. [CrossRef]
20. Henry BM, Lippi G, Plebani M. Letter to the editor. *Clin Chem Lab Med*. 2020;58(7):1135-1138. [CrossRef]
21. Lu X, Zhang L, Du H, et al. SARS-CoV-2 infection in children. *N Engl J Med*. 2020;382(17):1663-1665. [CrossRef]
22. Henry BM, Benoit SW, de Oliveira MHS, et al. Laboratory abnormalities in children with mild and severe coronavirus disease 2019 (COVID-19): a pooled analysis and review. *Clin Biochem*. 2020;81:1-8. [CrossRef]
23. Li Y, Deng W, Xiong H, et al. Immune-related factors associated with pneumonia in 127 children with coronavirus disease 2019 in Wuhan. *Pediatr Pulmonol*. 2020;55(9):2354-2360. [CrossRef]
24. 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):115-120. [CrossRef]
25. Lu W, Yang L, Li X, et al. Early immune responses and prognostic factors in children with COVID-19: a single-centre retrospective analysis. *BMC Pediatr*. 2021;21(1):181. [CrossRef]