in vivo *36*: 2813-2822 (2022)
doi:10.21873/invivo.13019

Biomarker Changes in Pediatric Patients With COVID-19: A Retrospective Study from a Single Center Database

CRISTIAN NICOLAE SAVA[1,2*], TEODORA-MARIA BODOG[3*], LARISA ROXANA NIULAS[1,2], ALIN REMUS IUHAS[1,2], CRISTIAN PHILLIP MARINAU[1,2], NICOLETA NEGRUT[4], ANDREEA BIANCA BALMOS[1,2*], BIANCA PASCA[5*], NADINNE ALEXANDRA ROMAN[6] and CARMEN DELIA NISTOR-CSEPPENTO[4]

[1]*Department of Medical Disciplines, Faculty of Medicine and Pharmacy, University of Oradea, Oradea, Romania;*
[2]*Department of Pediatrics, County Clinical Emergency Hospital of Oradea, Oradea, Romania;*
[3]*Faculty of Medicine and Pharmacy, University of Oradea, Oradea, Romania;*
[4]*Department of Psycho-neuroscience and Recovery, Faculty of Medicine and Pharmacy, University of Oradea, Oradea, Romania;*
[5]*Department of Pharmacy, Faculty of Medicine and Pharmacy, University of Oradea, Oradea, Romania;*
[6]*Faculty of Medicine, Transilvania University of Brasov, Brasov, Romania*

Abstract. *Background/Aim: The novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is responsible for generating a global pandemic with deadly consequences and life changes worldwide. With the appearance of the new variants of the virus, clinical manifestations have been reported in the pediatric population, some with severe evolution. The aim of this study was to identify the laboratory parameters necessary to establish an effective therapy. Patients and Methods: In the period from August 2020 to September 2021, 234 pediatric patients met the inclusion criteria and were selected for the study. After confirming the COVID-19 diagnosis, laboratory parameters were analyzed and compared to the severity of the illness. Results: Thrombocytopenia (p<0.001), leukocytosis (p<0.001), and lymphopenia (p<0.001) correlated with the severity of the disease. Also, D-dimer values were closely monitored due to the high association of this parameter with an unsatisfactory prognosis and a severe form of the disease. Conclusion: The D-dimer values and complete blood count are useful parameters in COVID-19 evaluation in children.*

*These Authors contributed equally to this work.

Correspondence to: Nicoleta Negrut, Universității street, no. 1, Oradea, Bihor region, 410087, Romania. Tel: +40 743139173, e-mail: lnm_n10@yahoo.com; Andreea Balmos, Universității street, no. 1, Oradea, Bihor region, 410087 Romania. Tel: +40 733399686, e-mail: andreeabalmos@gmail.com

Key Words: SARS-CoV-2, pediatric, leukocytosis, D-dimers, COVID-19.

The first cases of atypical pneumonia with uncommon manifestations and evolution emerged three years ago, on 31st December 2019, in Hubei Province, China (1). The inability of public health organizations to contain it and restrict its global spread made it a public health emergency of international concern on the 10th of January 2020, when the World Health Organization (WHO) announced more cases of pneumonia of unknown etiology in Wuhan (2).

During this pandemic, naturally, many mutations emerged. The variants of concern (VOC) include the Alpha variant (firstly identified in Great Britain, in December 2020); Beta (the first case reported in December 2020 in South Africa); Gamma (first reported on January 2021, in Brazil); Delta (reported in India, in December 2020) and last, but not least, Omicron (identified in multiple African countries in November 2021) (3). These viral mutations are not unusual, but they may represent particularities of the virus that must be examined and followed over time (4). The VOC, constitute public health problems regarding transmission, clinical presentations, and treatment (5). Some of them have changed in the incubation period, while others have more frequent fatal evolutions. The modifications may appear both in pediatric and adult patients, but the immediate and long-term effects were different depending on the mutations (6).

Knowledge of the virus' capacity, mutation rate, and adaptability are continually expanding. Medical literature claims that around two viral variants have appeared per

month, globally (7-10). The possibility of the emergence of new, more contagious, or dangerous variants remains an unsolved problem.

The infection rate has varied over the years, and five peaks are currently recognized in Romania. During the fourth and fifth waves, the Delta variant was responsible for the most severe form of the disease, requiring hospitalization. The infection with the Delta variant is correlated with severe cases and appears to be globally dominant since June 2020. From December 2019 to the present day, more than 554,466,955 cases including over 6,300,000 deaths have been confirmed worldwide. In Romania, until July 2022, approximately 2,919,461 cases of SARS-CoV-2 infection have been confirmed, including 65,739 deaths. Approximately 15% of these reported cases of COVID-19 were pediatric patients (11-13). In the case of children, COVID-19 can manifest at any age, and the incidence of it increases with age.

Clinically, COVID-19 is manifested by a diversity of symptoms, the most frequent being fever and respiratory symptoms (14). Cardiovascular, hematological, central nervous, gastrointestinal (GI), hepatobiliary, and renal systems are all affected by COVID-19, due to the presence of angiotensin-converting enzyme 2 (ACE2) receptors in all these organs (15-18). Approximately one-third of all cases are clinically asymptomatic (19), but the rest are classified as mild, moderate, severe, and critical. Pediatric patients present with milder forms of COVID-19, compared to adults (14); however, there are studies which suggest a significant deterioration in children than initially reported and anticipated (20, 21) (Figure 1). Clinical manifestations in most pediatric cases are not so different compared to those reported in adults. In fewer cases, the literature mentions some other, less common signs such as crepitations, sputum, abdominal pain, lymphadenopathy, cyanosis, and hypoxemia (22). Other, rare complications were reported, including purpuric rashes, a multisystem inflammatory syndrome in children (MIS-C), and chilblain-like lesions (CLLS) (23-27).

Together with clinical manifestations, it is mandatory to assess laboratory markers, which change according to the setting and the development of the inflammatory process. An increase in D-dimers, C-reactive protein and white blood cells, neutrophils, and lymphocyte count is expected in patients infected with SARS-CoV-2 (28). The study of Alkan *et al*. (2021) involving 550 pediatric cases of COVID-19, presented the analysis of a complete laboratory biomarker panel and indicated all the possible and eventual changes in biomarkers upon infection with SARS-CoV-2. They reported decreases in the levels of lymphocytes (36%), leukocytes (19.2%), and a significant increase in D-Dimers (17.9%) and reactive C protein (20.7%) (28).

A few studies showed white blood cell (WBC) elevation whereas others reported a decrease or no change (23, 29-38). In terms of lymphocytes, normal or elevated levels were reported, whereas reduced levels were scarcely reported (23, 29-38). The study by Zheng *et al*. (2020), on a group of 25 children in China, determined median values for WBCs to be 6.2×10^9/l (reference values: $4.5\text{-}11\times10^9$/l).

The meta-analysis by Qui *et al*. (2021), which evaluated 37 articles, totaling 2,874 children with COVID-19, found that the most frequently altered laboratory markers were lymphopenia (25 studies), leukopenia (27 studies), high reactive protein C (24 studies), high alanine transaminase (21 studies) and leukocytosis with high aspartate aminotransferase (both found in 19 studies); the D-dimer increase was found in a total of 11 studies (39).

The aim of this study was to present and discuss several laboratory markers that may influence the prognosis of pediatric patients with SARS-CoV-2 infection and identify the specific parameters to be assessed for the clinician to adopt a more appropriate course of therapy.

Patients and Methods

Protocol. A cohort study, including cases from August 2020 to November 2021, was performed in the Pediatric Unit of Dr. Gavril Curteanu, Municipal Hospital, Oradea, Romania. The hospital was a COVID-19 support unit during that period, treating only confirmed cases of SARS-CoV-2 infection. A total of 234 cases were selected for inclusion in this study.

The inclusion criterion was the positive diagnosis of COVID-19, while cases with oncological and hematological comorbidities were excluded (Figure 2).

The diagnosis of the virus was performed by detecting the antigen (rapid test) or the ribonucleic acid (RNA) [Real-time polymerase chain reaction (RT-PCR) assay] of SARS-CoV-2. All samples were collected within the first two hours following admission to the hospital. A Rapid Antigen kit (DDS DIAGNOSTIC, Bucharest, Romania) was used for the antigen detection of SARS-CoV-2. The kit contains anti-SARS-CoV-2 antibodies for the capture of the virus and anti-SARS-CoV-2 antibodies for detection. The test is performed with a nasopharyngeal sample and has a relative sensitivity of 98.77% and relative specificity of 99.03% (40). For the RT-PCR, the AllplexTM 2019-nCoV Assay was used, with the amplifier CFX96 Real-time System with the extractor SEEGENE NIMBUS, See-gene South Korea. The test had a sensitivity of 99.95% and a specificity of 100% (41).

Complete blood count from venous blood samples was measured using the DxH 900 Hematology Analyzer, Beckman Coulter (Brea, CA, USA).

D-dimers in plasma samples were analyzed using the DIAGON XXL Analyzer, Diagon (Budapest, Hungary), with normal values in the range of 0-198 ng/ml.

Severe forms of COVID-19 in children were defined according to the Romanian National Protocol for the treatment of infection with SARS-CoV-2 (42) as follows:

1. Asymptomatic form: identification of viral antigen or RNA SARS-CoV-2 in the nasopharyngeal sample.
2. Mild form: general and/or respiratory tract features, without pulmonary involvement.
3. Moderate form: general and/or respiratory tract features, with pulmonary involvement (clinical or imaging), with oxygen saturation (SpO2) ≥94% on room air.

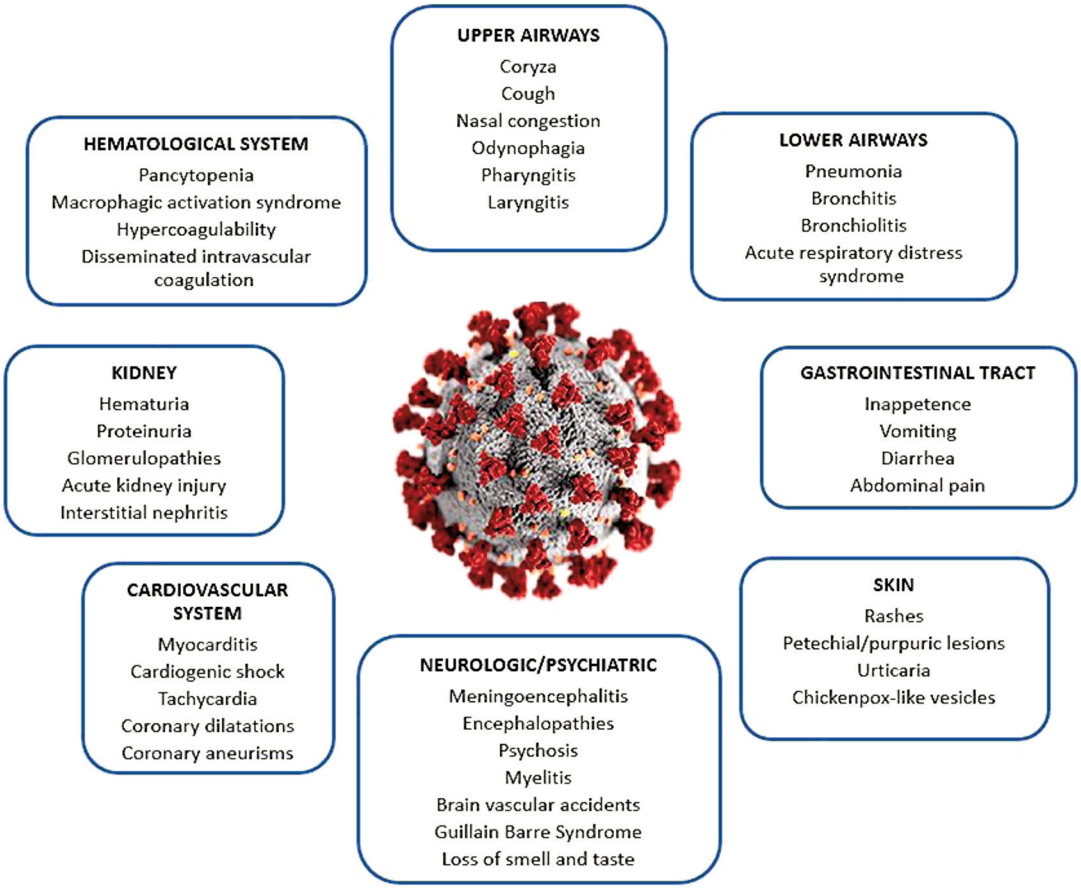

Figure 1. *Symptoms commonly found in pediatric patients*.

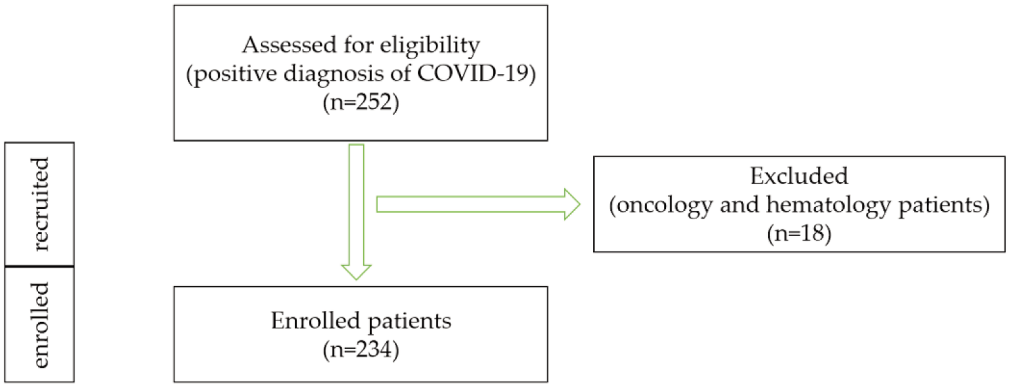

Figure 2. *CONSORT flow diagram of the study. n: Number of cases*.

4. Severe form: SpO2 <94% on room air, a ratio of arterial partial pressure of oxygen to fraction of inspired oxygen (PaO2/FiO2) <300 mm Hg, a respiratory rate >30 breaths/minute or lung infiltrates >50%.

Ethical statement. Written informed consent was obtained from the parents or legal guardians of the minors for all subjects involved in the study. The study was approved by the Ethics Commission of the County Clinical Emergency Hospital of Oradea, Bihor County,

Romania, (20335/23.06.2022) and respected the World Medical Association Code of Ethics (Declaration of Helsinki, 1967).

Statistical analysis. The software SPSS (Statistical Package for the Social Sciences), version 26, was used for statistical analysis and graphical representation. The comparison between the means of the different parameters was performed using the ANOVA test. Univariate analysis of variance (Levene's test) was used to examine the homogeneity of the dispersion of the samples if the ANOVA test was statistically significant. The Hochberg GT2 test was performed as a *post-hoc* analysis to compare two-by-two groups. The values obtained for different parameters were considered primary data for performing the Bravais-Pearson correlation coefficient (r). Its interpretation has value only in cases with $p<0.05$. Chi-squared test was used for comparing frequencies or proportions for two or more samples. Statistical significance was considered for a p-value ≤0.05.

Results

In the chosen period (August 2020 – November 2021) 252 children were declared eligible. Of these, 18 patients were excluded due to exclusion criteria. A total number of 234 children were finally included in the study. The group was relevant with a probability of 95%. The ratio of males to females was 1.08:1 ($p=0.513$). The study included pediatric patients with ages 0 to 17 years old, with a median age of 7.38±6.13 years old (Table I). The analysis of the disease manifestation in the current group underlined the presence of a milder form of infection ($p<0.001$) (Figure 3). Hematological alterations encountered during COVID-19 in the cohort are presented in Table II.

Leukocytosis was significantly more common in the severe form of the disease, ($p<0.001$). Leukopenia was more frequent in the age group over 12 years of age ($p<0.05$). No statistically significant correlation between leukopenia and disease manifestation was found ($p=0.065$).

Lymphopenia showed a significant correlation with the disease form, being more frequent in the severe form of the disease ($p<0.001$, r=0.324). Neutrophilia occurred more often in the age group over 12 years ($p=0.040$). No statistically significant associations were observed between neutrophilia and the form of the disease. The value of neutrophil to lymphocyte (NLR) index over 3 was often encountered in patients over 12 years old ($p<0.001$). Thrombocytopenia had a statistically significant correlation with the severe form of the illness ($p<0.001$, r=0.256), especially in the age group above 12 years old children ($p=0.004$, r=0.186). Thrombocytosis was common in infants ($p=0.016$). A statistically significant correlation was identified between thrombocytosis and the disease form, being more frequent in the severe form ($p<0.001$, r=0.345).

The mean value for D-dimers in the study group was 256.20±1,042.70 ng/ml, with a minimum of 20 ng/ml and a maximum of 4,700 ng/ml. The values of D-dimers at the four age categories were the following: 179.85±519.81 ng/ml (group <1 year), 150.37±213 ng/ml (group 1-4 years), 152.50±285.66 ng/ml (group 4-12 years), and 439.72±1,706.24 ng/ml (group 12-18 years).

The ANOVA test revealed a significant difference between these values, $F(3, 233)=3.057$; $p=0.029$. To choose a test post-hoc, the homogeneity of the dispersion through the Levene test was verified. According to the statistical analysis made through this test, the dispersions between these four groups were considered equal ($p=0.720$).

In the cases with equal dispersions and an unequal subject number, a post-hoc Hochberg GT2 test was performed (Table III). The *post-hoc* analysis, applied to compare the groups

Table I. *Demographic characteristics of the study group*.

Parameter	Study group	
Age groups	M±SD	
<1 year	0.66±0.22	
1-4 years	2.00±0.94	
4-12 years	7.28±2.39	
12-18 years	14.90±1.65	
	N (%)	*p*-Value
<1 year	61 (26.1)	0.081
1-4 years	43 (18.4)	
4-12 years	51 (21.8)	
12-18 years	79 (33.8)	
Sex		
Female	112 (47.8)	0.513
Residence		
Urban	109 (46.69)	0.295

M: Median, SD: standard deviation; N: total number.

Table II. *Hematological changes in the study group*.

Parameter	Study group
Leukocyte modifications	N (%)
Leukocytosis	11 (4.7)
Leukopenia	48 (20.51)
Neutrophilia	25 (10.68)
Neutropenia	41 (17.5)
Lymphopenia	35 (14.95)
NLR*	37 (15.8)
Platelet modifications	
Thrombocytopenia	12 (5.1)
Thrombocytosis	16 (6.8)
Erythrocyte modifications	
Anemia	19 (8.1)
Hypochromic microcytic anemia	4 (1.7)
Normochromic, normocytic anemia	15 (6.4)

N: Total number; NLR: neutrophil-lymphocyte ratio, *NLR >3.

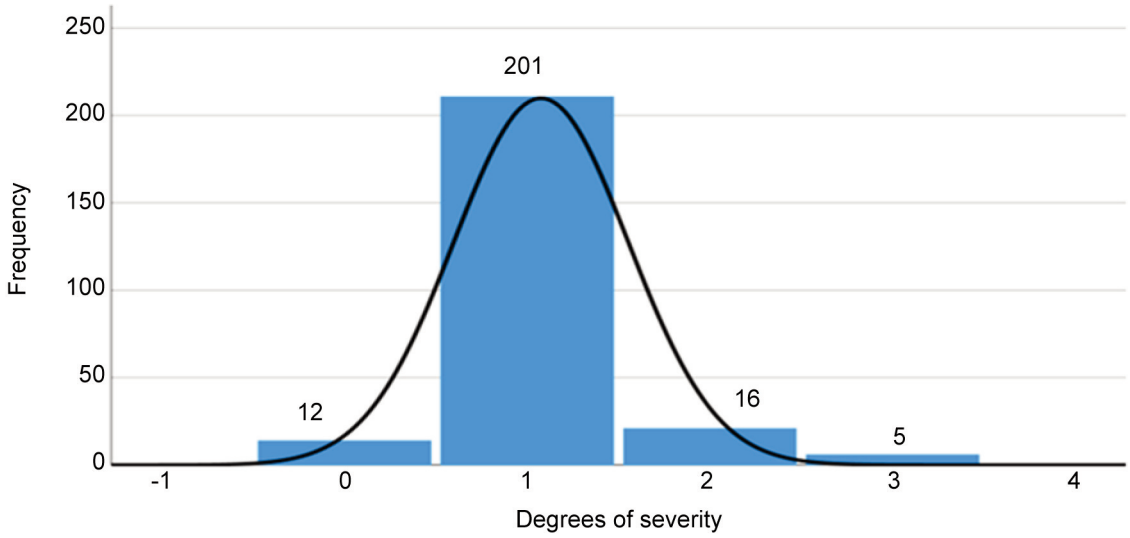

Figure 3. *Form of disease distribution of study group. 0 – asymptomatic forms; 1 – mild forms; 2 – moderate forms; 3 – severe forms.*

Table III. *D-dimer variations according to the ages.*

Multiple comparisons
Dependent variable: D-dimer values
Hochberg

(I) Age (year)	(J) Age (year)	MD (I-J)	SE	*p*-Value	95%CI	
					Lower bound	Upper bound
<1	1-4	–0.051	0.068	0.973	–0.23	0.13
	4-12	–0.032	0.064	0.997	–0.20	0.14
	12-18	–0.162*	0.058	0.033	–0.32	–0.01
1-4	<1	0.051	0.068	0.973	–0.13	0.23
	4-12	0.018	0.070	0.999	–0.17	0.20
	12-18	–0.112	0.064	0.409	–0.28	0.06
4-12	<1	0.032	0.064	0.997	–0.14	0.20
	1-4	–0.018	0.070	0.999	–0.20	0.17
	12-18	–0.130	0.061	0.189	–0.29	0.03
12-18	<1	0.162*	0.058	0.033	0.01	0.32
	1-4	0.112	0.064	0.409	–0.06	0.28
	4-12	0.130	0.061	0.189	–0.03	0.29

MD: Mean difference; SE: standard error; CI: confidence interval. *The mean difference is significant at the 0.05 level.

two by two, showed that there was a statistically significant difference between the value of the mean and standard deviation of D-dimers registered for the patients with ages below 1-year-old *versus* those between 12 and 18 years old (p=0.033). Other statistically significant differences between the values of the D-dimers registered in the other groups were not observed.

Discussion

During the COVID-19 pandemic years, pediatric patients were initially neglected. However, with the occurrence of more VOCs, particularities of pediatric patients were inevitable. The COVID-19 pandemic still poses a threat and there is still much research to perform to fully comprehend

the magnitude of this virus and its implications and manifestations in pediatric patients. In Romania, there are still few studies on the association of laboratory findings with COVID-19 manifestations.

The values of NLR, leukocytes, and neutrophils, along with the inflammatory syndrome are paraclinical markers used early to identify the etiologies of various infectious diseases. Their elevation indicates bacterial infection, before obtaining results from cultures or specific tests for microorganisms' identification. However, when interpreting the results, it is necessary to consider the variations in lymphocytes and neutrophils according to the age of the child.

Leukopenia can occur in the case of infections with certain types of viruses, or in the case of severe bacterial infections. The decrease in the number of leukocytes impairs the defence of the body by depriving it of specialized immune cells. Leukopenia has been reported in cases of coronavirus infection causing severe disease in humans, and less in common human coronaviruses, which cause common flu-like symptoms (43). In this study, most cases (48, 20.51%) and especially those in the age group above 12 years old presented leukopenia, which may be due to the presence of puberty (44). Our results are consistent with those in the medical literature. A statistically significant correlation could not be established between leukopenia and the manifestation of the disease. The study by Bari *et al*., conducted from May to June 2021, on 83 pediatric patients, showed that changes in leukopenia did not represent a useful prognostic factor (45). However, in this study, leukocytosis appeared to be strongly correlated to the disease form. Currently, the literature lacks sufficient information regarding the role of leukopenia as a prognostic factor for pediatric patients.

NLR is considered to reflect a poor outcome in pediatric patients. In the retrospective study of Ciccullo *et al*., conducted on 74 patients with COVID-19, in March 2020, a median value of NLR of around 4.5 was correlated with the severity of the illness and the poor evolution (46). In this study, NLR values above 3 were mainly observed in the group aged above 12 years old, again an aspect which varies from individual to individual and which might be dependent on the onset of puberty (44). NLR is a parameter by which clinicians can evaluate the stress on the body at a current time (47). Puberty, through the hormonal storm it brings, results in increased stress to the human body, which is added to that of infection with an aggressive virus that can cause a storm of cytokines and low-molecular-weight proteins whose functions are often mimicked by the hormones.

Lymphopenia is usually observed in viral infections due to the action of the virus on the immune system and the destruction of CD4+ cells, which results in the reduction in the levels of cytokines that protect against various pathogens (48). The study of Liu *et al*. (2021) underlined the significant difference between children and adults, where children below 12 years of age presented more often with lymphopenia in the first days of the disease, possibly because of the different immunological responses (48). Lymphopenia was also registered in this study group at a rate of approximately 35%. There is a lack of studies on lymphocytic changes, which does not allow comparison of the data from the present study to those of the literature; there are only two studies (49, 50) in small cohorts of 66 and 52 patients. The study of Guang *et al*., on 66 cases, reported no significant changes in lymphocytic values used to evaluate prognosis or to differentiate from mild to moderate or severe illness (49). In this study, significant lymphopenia was present in the severe form of the disease. On the other hand, the study of Rezaei *et al*., on 52 patients, reported an increase in lymphocytic values in opposition to the previously mentioned study, in moderate and mild cases (50). In the study by Alkan *et al*., (2021), performed on a pediatric group of 633 patients with COVID-19 between March 2020 to January 2021, lymphopenia was also found present in approximately 36% of cases (28). The difference between the present study and the study of Guang *et al*., can be explained by the small number of patients and the age difference.

Neutropenia is frequently associated with viral infections due to the viral-induced capacity of the neutrophils to redistribute from the main circulation to marginal areas, which occurs after the first 1-2 days of the infection and remains up to 8-9 days (51). Even so, there still is a lack of data in terms of its correlation with disease severity. Neutropenia was detected in the present study at an increased rate of 17.5%. In the medical literature, the rate of neutropenia in COVID-19 pediatric patients was found to be approximately 7.6% (28, 52, 53). In this study, neutrophilia was present in approximately 10.68% of the analyzed cases, mostly in patients aged above 12 years, and it was not correlated to the disease form.

The elevation of D-dimers was one of the frequently followed biomarkers in adults and an indicator of the risk of development of various coagulopathies. The inclusion of it in the guidelines, for pediatric COVID-19 patients, was made later during the pandemic period (44, 54, 55). D-dimer values ranged from 20-4,700 ng/ml in the present study. The meta-analysis of Zhang *et al*. (2021), which included a total of 5,557 articles extracted from the Web of Science, revealed that the D-dimer increase can separate fatal cases of COVID-19 from moderate or mild cases, with a sensitivity of 77% and a specificity of 71% (56). Their study suggests that D-dimer values can predict prognosis, even though the patients considered were adults, the increase reported by us was also associated with a poor prognosis. In the study of Alkan *et al*. (2021), D-dimers were found to be elevated in approximately 17.9% of patients (28). In the present study, elevated values of D-dimers were statistically significantly more present in the

age group above 12 years old, with small differences from the other age groups. However, this difference may be attributed also to the continuously moving curve of puberty. Bellis *et al*., (2006), traced the changing curve of puberty from the 19th century to the present and revealed a decrease in the age of puberty, as well as the appearance of specific body changes as early as 11 years old, while in the 19th century these changes occurred only at the age of 16-17 years (44).

In the present study, thrombocytopenia was strongly correlated with the severe form of COVID-19, especially in children over 12 years of age. Thrombocytosis was found more frequently in infants being correlated with severe forms. Thus, 6.8% of cases with thrombocytosis and 5.1% with thrombocytopenia were recorded. Considering the medical literature, these parameters apparently do not differentiate between the form or severity of the disease. These changes might be associated with the treatment with heparin, and thrombocytopenia has been mostly reported in pediatric patients who develop MIS-C (57). Evens so, there is a lack of findings in the medical literature regarding these parameters, and there are only a few case reports regarding thrombocytosis and thrombocytopenia (57-61).

Some particularities can be discussed from a strengths and weaknesses point of view. A weak point of this study is that the effect of different viral variants on the monitored laboratory parameters was not examined. In terms of strengths, the total number of patients was significant, as well as the number of patients included in the different age groups. This study is the first Romanian study to focus on changes in laboratory biomarkers in pediatric patients with COVID-19.

The risk of bias was not evaluated in the current study, but the authors report no bias or conflict of interest. All the studies, reviews, and meta-analyses included in this study have been qualitatively assessed and the considered sources have been checked multiple times for bias risks and conflict of interests.

Future research and re-evaluation of the presented criteria may include a larger number of patients from different hospitals. Also, identification of the virus variant should be included in future studies allowing the establishment of separate blood panels for each variant. These panels may determine whether the patient may be placed at risk for the development of severe disease with future complications.

Conclusion

COVID-19 represents a major health problem. Therefore, it still requires much study and protocol development. The present study recommends monitoring D-dimers, neutrophils, platelets, and lymphocytes to determine disease prognosis. NLR may be a potential predictor of poor prognosis. The other laboratory markers have not been clinically correlated to the disease form but following these markers should be enough for a clinician to adapt the treatment scheme and evaluate the disease prognosis.

Conflicts of Interest

The Authors do not have any conflicts of interest to declare in relation to this study.

Authors' Contributions

Conceptualization, Cristian Nicolae Sava; Data curation, Larisa Roxana Niulaș; Investigation, Cristian Nicolae Sava, Larisa Roxana Niulaș, Alin Remus Iuhas, Cristian Phillip Marinau, Bianca Pasca and Andreea Bianca Balmos, Nadinne Alexandra Roman; Methodology, Cristian Nicolae Sava; Supervision, Nicoleta Negruț; Validation, Carmen Delia Nistor-Cseppento; Visualization, Carmen Delia Nistor-Cseppento; Writing – original draft, Teodora-Maria Bodog, Nicoleta Negruț and Carmen Delia Nistor-Cseppento; Writing – review & editing, Nicoleta Negruț. All Authors have read and agreed to the published version of the manuscript.

Acknowledgements

The Authors express their gratitude to the University of Oradea, which supported APC.

Funding

APC was supported by the University of Oradea, Oradea, Romania through an internal project.

References

1 Chan JF, Yuan S, Kok KH, To KK, Chu H, Yang J, Xing F, Liu J, Yip CC, Poon RW, Tsoi HW, Lo SK, Chan KH, Poon VK, Chan WM, Ip JD, Cai JP, Cheng VC, Chen H, Hui CK and Yuen KY: A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster. Lancet *395(10223)*: 514-523, 2020. PMID: 31986261. DOI: 10.1016/S0140-6736(20)30154-9

2 Awadasseid A, Wu Y, Tanaka Y and Zhang W: Initial success in the identification and management of the coronavirus disease 2019 (COVID-19) indicates human-to-human transmission in Wuhan, China. Int J Biol Sci *16(11)*: 1846-1860, 2020. PMID: 32398954. DOI: 10.7150/ijbs.45018

3 Volz E, Mishra S, Chand M, Barrett JC, Johnson R, Geidelberg L, Hinsley WR, Laydon DJ, Dabrera G, O'Toole Á, Amato R, Ragonnet-Cronin M, Harrison I, Jackson B, Ariani CV, Boyd O, Loman NJ, McCrone JT, Gonçalves S, Jorgensen D, Myers R, Hill V, Jackson DK, Gaythorpe K, Groves N, Sillitoe J, Kwiatkowski DP, COVID-19 Genomics UK (COG-UK) consortium, Flaxman S, Ratmann O, Bhatt S, Hopkins S, Gandy A, Rambaut A and Ferguson NM: Assessing transmissibility of SARS-CoV-2 lineage B.1.1.7 in England. Nature *593(7858)*: 266-269, 2021. PMID: 33767447. DOI: 10.1038/s41586-021-03470-x

4 Behl T, Kaur I, Aleya L, Sehgal A, Singh S, Sharma N, Bhatia S, Al-Harrasi A and Bungau S: CD147-spike protein interaction in COVID-19: Get the ball rolling with a novel receptor and

therapeutic target. Sci Total Environ *808*: 152072, 2022. PMID: 34863742. DOI: 10.1016/j.scitotenv.2021.152072

5 Negrut N, Codrean A, Hodisan I, Bungau S, Tit DM, Marin R, Behl T, Banica F, Diaconu CC and Nistor-Cseppento DC: Efficiency of antiviral treatment in COVID-19. Exp Ther Med *21(6)*: 648, 2021. PMID: 33968179. DOI: 10.3892/etm.2021.10080

6 Moga TD, Nistor-Cseppento CD, Bungau SG, Tit DM, Sabau AM, Behl T, Nechifor AC, Bungau AF and Negrut N: The effects of the 'catabolic crisis' on patients' prolonged immobility after COVID-19 Infection. Medicina (Kaunas) *58(6)*: 828, 2022. PMID: 35744091. DOI: 10.3390/medicina58060828

7 Worobey M, Pekar J, Larsen BB, Nelson MI, Hill V, Joy JB, Rambaut A, Suchard MA, Wertheim JO and Lemey P: The emergence of SARS-CoV-2 in Europe and North America. Science *370(6516)*: 564-570, 2020. PMID: 32912998. DOI: 10.1126/science.abc8169

8 Duchene S, Featherstone L, Haritopoulou-Sinanidou M, Rambaut A, Lemey P and Baele G: Temporal signal and the phylodynamic threshold of SARS-CoV-2. Virus Evol *6(2)*: veaa061, 2020. PMID: 33235813. DOI: 10.1093/ve/veaa061

9 Harvey WT, Carabelli AM, Jackson B, Gupta RK, Thomson EC, Harrison EM, Ludden C, Reeve R, Rambaut A, COVID-19 Genomics UK (COG-UK) Consortium, Peacock SJ and Robertson DL: SARS-CoV-2 variants, spike mutations and immune escape. Nat Rev Microbiol *19(7)*: 409-424, 2021. PMID: 34075212. DOI: 10.1038/s41579-021-00573-0

10 Kabir MT, Uddin MS, Hossain MF, Abdulhakim JA, Alam MA, Ashraf GM, Bungau SG, Bin-Jumah MN, Abdel-Daim MM and Aleya L: nCOVID-19 pandemic: from molecular pathogenesis to potential investigational therapeutics. Front Cell Dev Biol *8*: 616, 2020. PMID: 32754599. DOI: 10.3389/fcell.2020.00616

11 Statista: Distribution of coronavirus cases in Italy as of July 6, 2022, by age group. Available at: https://www.statista.com/statistics/1103023/coronavirus-cases-distribution-by-age-group-italy/ [Last accessed on July 17, 2022]

12 Statista: Age distribution of coronavirus (COVID-19) cases in South Korea as of July 1, 2022. Available at: https://www.statista.com/statistics/1102730/south-korea-coronavirus-cases-by-age/ [Last accessed on July 17, 2022]

13 Dawood FS, Porucznik CA, Veguilla V, Stanford JB, Duque J, Rolfes MA, Dixon A, Thind P, Hacker E, Castro MJE, Jeddy Z, Daugherty M, Altunkaynak K, Hunt DR, Kattel U, Meece J and Stockwell MS: Incidence rates, household infection risk, and clinical characteristics of SARS-CoV-2 infection among children and adults in Utah and New York City, New York. JAMA Pediatrics *176(1)*: 59-67, 2022. DOI:10.1097/inf.0000000000003408

14 Irfan O, Muttalib F, Tang K, Jiang L, Lassi ZS and Bhutta Z: Clinical characteristics, treatment and outcomes of paediatric COVID-19: a systematic review and meta-analysis. Arch Dis Child, 2021. PMID: 33593743. DOI: 10.1136/archdischild-2020-321385

15 Cascella M, Rajnik M, Aleem A, Dulebohn SC and Napoli RD: Features, evaluation, and treatment of coronavirus (COVID-19). Treasure Island, FL, USA, StatPearls Publishing, 2022.

16 Gheorghe G, Ilie M, Bungau S, Stoian AMP, Bacalbasa N and Diaconu CC: Is there a relationship between COVID-19 and hyponatremia? Medicina (Kaunas) *57(1)*: 55, 2021. PMID: 33435405. DOI: 10.3390/medicina57010055

17 Sharma P, Behl T, Sharma N, Singh S, Grewal AS, Albarrati A, Albratty M, Meraya AM and Bungau S: COVID-19 and diabetes: Association intensify risk factors for morbidity and mortality. Biomed Pharmacother *151*: 113089, 2022. PMID: 35569351. DOI: 10.1016/j.biopha.2022.113089

18 Behl T, Kaur I, Bungau S, Kumar A, Uddin MS, Kumar C, Pal G, Sahil, Shrivastava K, Zengin G and Arora S: The dual impact of ACE2 in COVID-19 and ironical actions in geriatrics and pediatrics with possible therapeutic solutions. Life Sci *257*: 118075, 2020. PMID: 32653522. DOI: 10.1016/j.lfs.2020.118075

19 Jiang F, Deng L, Zhang L, Cai Y, Cheung CW and Xia Z: Review of the clinical characteristics of Coronavirus disease 2019 (COVID-19). J Gen Intern Med *35(5)*: 1545-1549, 2020. PMID: 32133578. DOI: 10.1007/s11606-020-05762-w

20 Siegel DA, Reses HE, Cool AJ, Shapiro CN, Hsu J, Boehmer TK, Cornwell CR, Gray EB, Henley SJ, Lochner K, Suthar AB, Lyons BC, Mattocks L, Hartnett K, Adjemian J, van Santen KL, Sheppard M, Soetebier KA, MAPW1, Logan P, Martin M, Idubor O, Natarajan P, Sircar K, Oyegun E, Dalton J, Perrine CG, Peacock G, Schweitzer B, Morris SB and Raizes E: Trends in COVID-19 cases, emergency department visits, and hospital admissions among children and adolescents aged 0-17 years - United States, August 2020-August 2021. MMWR Morb Mortal Wkly Rep *70(36)*: 1249-1254, 2021. PMID: 34499628. DOI: 10.15585/mmwr.mm7036e1

21 Bhuiyan MU, Stiboy E, Hassan MZ, Chan M, Islam MS, Haider N, Jaffe A and Homaira N: Epidemiology of COVID-19 infection in young children under five years: A systematic review and meta-analysis. Vaccine *39(4)*: 667-677, 2021. PMID: 33342635. DOI: 10.1016/j.vaccine.2020.11.078

22 de Souza TH, Nadal JA, Nogueira RJN, Pereira RM and Brandão MB: Clinical manifestations of children with COVID-19: A systematic review. Pediatr Pulmonol *55(8)*: 1892-1899, 2020. PMID: 32492251. DOI: 10.1002/ppul.24885

23 Colonna C, Restano L, Monzani N, Zussino M, Ponziani A, Cambiaghi S and Cavalli R: Rare and common manifestations of COVID-19 in children. JEADV Clinical Practice *1(1)*: 21-30, 2022. DOI: 10.1002/jvc2.10

24 Blatz AM, Oboite M, Chiotos K, Castelo-Soccio L, Odom John AR and Children's Hospital of Philadelphia MIS-C Research Collaborative: Cutaneous Findings in SARS-CoV-2-Associated Multisystem Inflammatory Disease in Children. Open Forum Infect Dis *8(3)*: ofab074, 2021. PMID: 33778091. DOI: 10.1093/ofid/ofab074

25 Yozgat CY, Uzuner S, Bursal Duramaz B, Yozgat Y, Erenberk U, Iscan A and Turel O: Dermatological manifestation of pediatrics multisystem inflammatory syndrome associated with COVID-19 in a 3-year-old girl. Dermatol Ther *33(4)*: e13770, 2020. PMID: 32500647. DOI: 10.1111/dth.13770

26 Mazori DR, Derrick KM, Kapoor U, Haribhai M, Gist RE and Glick SA: Perineal desquamation: An early sign of the Kawasaki disease phenotype of MIS-C. Pediatr Dermatol *38(1)*: 253-256, 2021. PMID: 33222290. DOI: 10.1111/pde.14462

27 Sert A: A response to "Mucocutaneous disease and related clinical characteristics in hospitalized children and adolescents with COVID-19 and multisystem inflammatory syndrome in children". J Am Acad Dermatol *84(6)*: e301-e302, 2021. PMID: 33677002. DOI: 10.1016/j.jaad.2021.02.076

28 Alkan G, Sert A, Emiroglu M, Tuter Oz SK and Vatansev H: Evaluation of hematological parameters and inflammatory markers in children with COVID-19. Ir J Med Sci *191(4)*: 1725-1733, 2022. PMID: 34529237. DOI: 10.1007/s11845-021-02762-5

29 Wang D, Ju XL, Xie F, Lu Y, Li FY, Huang HH, Fang XL, Li YJ, Wang JY, Yi B, Yue JX, Wang J, Wang LX, Li B, Wang Y, Qiu BP, Zhou ZY, Li KL, Sun JH, Liu XG, Li GD, Wang YJ, Cao AH and Chen YN: Clinical analysis of 31 cases of 2019 novel coronavirus infection in children from six provinces (autonomous region) of northern China. Zhonghua Er Ke Za Zhi *58(4)*: 269-274, 2020. PMID: 32118389. DOI: 10.3760/cma.j.cn112140-20200225-00138

30 Xia W, Shao J, Guo Y, Peng X, Li Z and Hu D: Clinical and CT features in pediatric patients with COVID-19 infection: Different points from adults. Pediatr Pulmonol *55(5)*: 1169-1174, 2020. PMID: 32134205. DOI: 10.1002/ppul.24718

31 Sun D, Li H, Lu XX, Xiao H, Ren J, Zhang FR and Liu ZS: Clinical features of severe pediatric patients with coronavirus disease 2019 in Wuhan: a single center's observational study. World J Pediatr *16(3)*: 251-259, 2020. PMID: 32193831. DOI: 10.1007/s12519-020-00354-4

32 Liu W, Zhang Q, Chen J, Xiang R, Song H, Shu S, Chen L, Liang L, Zhou J, You L, Wu P, Zhang B, Lu Y, Xia L, Huang L, Yang Y, Liu F, Semple MG, Cowling BJ, Lan K, Sun Z, Yu H and Liu Y: Detection of Covid-19 in children in early January 2020 in Wuhan, China. N Engl J Med *382(14)*: 1370-1371, 2020. PMID: 32163697. DOI: 10.1056/NEJMc2003717

33 Hu Z, Song C, Xu C, Jin G, Chen Y, Xu X, Ma H, Chen W, Lin Y, Zheng Y, Wang J, Hu Z, Yi Y and Shen H: Clinical characteristics of 24 asymptomatic infections with COVID-19 screened among close contacts in Nanjing, China. Sci China Life Sci *63(5)*: 706-711, 2020. PMID: 32146694. DOI: 10.1007/s11427-020-1661-4

34 Liu H, Liu F, Li J, Zhang T, Wang D and Lan W: Clinical and CT imaging features of the COVID-19 pneumonia: Focus on pregnant women and children. J Infect *80(5)*: e7-e13, 2020. PMID: 32171865. DOI: 10.1016/j.jinf.2020.03.007

35 Li Y, Guo F, Cao Y, Li L and Guo Y: Insight into COVID-2019 for pediatricians. Pediatr Pulmonol *55(5)*: E1-E4, 2020. PMID: 32187887. DOI: 10.1002/ppul.24734

36 Ji LN, Chao S, Wang YJ, Li XJ, Mu XD, Lin MG and Jiang RM: Clinical features of pediatric patients with COVID-19: a report of two family cluster cases. World J Pediatr *16(3)*: 267-270, 2020. PMID: 32180140. DOI: 10.1007/s12519-020-00356-2

37 Park JY, Han MS, Park KU, Kim JY and Choi EH: First pediatric case of Coronavirus disease 2019 in Korea. J Korean Med Sci *35(11)*: e124, 2020. PMID: 32193905. DOI: 10.3346/jkms.2020.35.e124

38 Cui Y, Tian M, Huang D, Wang X, Huang Y, Fan L, Wang L, Chen Y, Liu W, Zhang K, Wu Y, Yang Z, Tao J, Feng J, Liu K, Ye X, Wang R, Zhang X and Zha Y: A 55-day-old female infant infected with 2019 novel Coronavirus disease: Presenting with pneumonia, liver injury, and heart damage. J Infect Dis *221(11)*: 1775-1781, 2020. PMID: 32179908. DOI: 10.1093/infdis/jiaa113

39 Qi K, Zeng W, Ye M, Zheng L, Song C, Hu S, Duan C, Wei Y, Peng J, Zhang W and Xu J: Clinical, laboratory, and imaging features of pediatric COVID-19: A systematic review and meta-analysis. Medicine (Baltimore) *100(15)*: e25230, 2021. PMID: 33847620. DOI: 10.1097/MD.0000000000025230

40 Diagnostic, D.D.S. Test Antigen Covid-19 tampon nazofaringian. Available at: https://ddsdiagnostic.com/produs/kit-tesrapid-covid-19-antigen-tampon-nazofaringian/ [Last accessed on July 14, 2022]

41 US, F.D.A. AllplexTM 2019-nCoV Assay. Available at: https://www.fda.gov/media/137178/download [Last accessed on July 17, 2022]

42 Romanian Ministry of Public Health, Monitorul Oficial, Partea I nr. 978 din 13 octombrie 2021. Ordinul nr. 2.103/2021 privind modificarea anexei la Ordinul ministrului sănătăţii nr. 487/2020 pentru aprobarea protocolului de tratament al infecţiei cu virusul SARS-CoV-2. Available at: https://lege5.ro/Gratuit/he2dsnrrga3q/ordinul-nr-2103-2021-privind-modificarea-anexei-la-ordinul-ministrului-sanatatii-nr-487-2020-pentru-aprobarea-protocolului-de-tratament-al-infectiei-cu-virusul-sars-cov-2 [Last accessed on July 14, 2022]

43 Ceglie G, De Ioris MA, Mercadante S, Olivini N, Del Bufalo F, Marchesani S, Cocca F, Monteferrario E, Boccieri E, Pianese J and Palumbo G: Immune thrombocytopenia in a child with COVID-19: Is it the calm after the (cytokine) storm? Pediatr Blood Cancer *69(1)*: e29326, 2022. PMID: 34490996. DOI: 10.1002/pbc.29326

44 Bellis MA, Downing J and Ashton JR: Adults at 12? Trends in puberty and their public health consequences. J Epidemiol Community Health *60(11)*: 910-911, 2006. PMID: 17053275. DOI: 10.1136/jech.2006.049379

45 Bari A, Ch A, Bano I and Saqlain N: Is leukopenia and lymphopenia a characteristic feature of COVID-19 in children? Pak J Med Sci *37(3)*: 869-873, 2021. PMID: 34104180. DOI: 10.12669/pjms.37.3.3848

46 Ciccullo A, Borghetti A, Zileri Dal Verme L, Tosoni A, Lombardi F, Garcovich M, Biscetti F, Montalto M, Cauda R, Di Giambenedetto S and GEMELLI AGAINST COVID Group: Neutrophil-to-lymphocyte ratio and clinical outcome in COVID-19: a report from the Italian front line. Int J Antimicrob Agents *56(2)*: 106017, 2020. PMID: 32437920. DOI: 10.1016/j.ijantimicag.2020.106017

47 Toori KU, Qureshi MA, Chaudhry A and Safdar MF: Neutrophil to lymphocyte ratio (NLR) in COVID-19: A cheap prognostic marker in a resource constraint setting. Pak J Med Sci *37(5)*: 1435-1439, 2021. PMID: 34475926. DOI: 10.12669/pjms.37.5.4194

48 Goldenberg NA, Sochet A, Albisetti M, Biss T, Bonduel M, Jaffray J, MacLaren G, Monagle P, O'Brien S, Raffini L, Revel-Vilk S, Sirachainan N, Williams S, Zia A, Male C and Pediatric/Neonatal Hemostasis and Thrombosis Subcommittee of the ISTH SSC: Consensus-based clinical recommendations and research priorities for anticoagulant thromboprophylaxis in children hospitalized for COVID-19-related illness. J Thromb Haemost *18(11)*: 3099-3105, 2020. PMID: 33174388. DOI: 10.1111/jth.15073

49 Yang G, Feng F, Li X, Zhang T, Li X and Li B: Changes of lymphocyte subsets in patients with COVID-19 and clinical significance: a case-control observational study. Journal of Bio-X Research *4(1)*: 36-39, 2021. DOI: 10.1097/JBR.0000000000000089

50 Rezaei M, Marjani M, Mahmoudi S, Mortaz E and Mansouri D: Dynamic changes of lymphocyte subsets in the course of COVID-19. Int Arch Allergy Immunol *182(3)*: 254-262, 2021. PMID: 33498051. DOI: 10.1159/000514202

51 Liu L, She J, Bai Y and Liu W: SARS-CoV-2 infection: differences in hematological parameters between adults and children. Int J Gen Med *14*: 3035-3047, 2021. PMID: 34234532. DOI: 10.2147/IJGM.S313860

52 Martins MM, Prata-Barbosa A, Magalhães-Barbosa MC and Cunha AJLAD: Clinical and laboratory characteristics of SARS-

COV-2 infection in children and adolescents. Rev Paul Pediatr *39*: e2020231, 2020. PMID: 33206842. DOI: 10.1590/1984-0462/2021/39/2020231

53 Han YN, Feng ZW, Sun LN, Ren XX, Wang H, Xue YM, Wang Y and Fang Y: A comparative-descriptive analysis of clinical characteristics in 2019-coronavirus-infected children and adults. J Med Virol *92(9)*: 1596-1602, 2020. PMID: 32249943. DOI: 10.1002/jmv.25835

54 Chen ZM, Fu JF, Shu Q, Chen YH, Hua CZ, Li FB, Lin R, Tang LF, Wang TL, Wang W, Wang YS, Xu WZ, Yang ZH, Ye S, Yuan TM, Zhang CM and Zhang YY: Diagnosis and treatment recommendations for pediatric respiratory infection caused by the 2019 novel coronavirus. World J Pediatr *16(3)*: 240-246, 2020. PMID: 32026148. DOI: 10.1007/s12519-020-00345-5

55 Cui X, Zhang T, Zheng J, Zhang J, Si P, Xu Y, Guo W, Liu Z, Li W, Ma J, Dong C, Shen Y, Cai C and He S: Children with coronavirus disease 2019: A review of demographic, clinical, laboratory, and imaging features in pediatric patients. J Med Virol *92(9)*: 1501-1510, 2020. PMID: 32418216. DOI: 10.1002/jmv.26023

56 Zhan H, Chen H, Liu C, Cheng L, Yan S, Li H and Li Y: Diagnostic value of D-dimer in COVID-19: a meta-analysis and meta-regression. Clin Appl Thromb Hemost *27*: 10760296211010976, 2021. PMID: 33926262. DOI: 10.1177/10760296211010976

57 Saleh Z, Khera S and Gavvala S: Thrombocytosis: Not always reactive. J Investig Med *69(2)*: 632-633, 2021.

58 Kossiva L, Thirios A, Panagouli E, Panos A, Lampidi S, Bacopoulou F, Tsolia M and Tsitsika A: A case of COVID-19-related thrombocytopenia and leukopenia in an adolescent with mild symptoms. Children (Basel) *8(6)*: 509, 2021. PMID: 34208610. DOI: 10.3390/children8060509

59 Vadakkekara J, Mathew R and Khera S: COVID-19-associated immune thrombocytopenia in a toddler. Indian J Pediatr *89(6)*: 623, 2022. PMID: 35298772. DOI: 10.1007/s12098-022-04109-z

60 Ambike D and Bijarniya K: Asymptomatic pediatric Covid-19 presenting with thrombocytopenia – a rare finding. Indian J Pediatr *88(1)*: 103-104, 2021. PMID: 32857332. DOI: 10.1007/s12098-020-03482-x

61 Ötiken Arıkan K, Şahinkaya Ş, Böncüoğlu E, Kıymet E, Cem E, Akaslan Kara A, Bayram N and Devrim İ: Can hematological findings of COVID-19 in pediatric patients guide physicians regarding clinical severity? Turk J Haematol *38(3)*: 243-245, 2021. PMID: 33906331. DOI: 10.4274/tjh.galenos.2021.2021.0157

Received September 2, 2022
Revised October 7, 2022
Accepted October 11, 2022