HEMATOLOGICAL PARAMETERS IN CHILDREN WITH SEVERE ACUTE RESPIRATORY SYNDROME CORONAVIRUS 2 INFECTION

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HEMATOLOGIȘKI PARAMETRI KOD DECE SA INFEKCIJOM TEŠKOG AKUTNOG RESPIRATORNOG SINDROMA KORONA-VIRUS 2

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Summary
Introduction. Recently, there has been a need to use more readily available parameters to assess the severe acute respiratory syndrome coronavirus 2 infection in children. Material and Methods. A single-center retrospective study included 30 children with severe acute respiratory syndrome coronavirus 2 infection who were admitted to the Isolation Department of the Institute for Child and Youth Health Care of Vojvodina from April to September 2020. A complete blood count with differential was performed. Additionally, systemic inflammatory index, neutrophil-lymphocyte ratio and platelet-lymphocyte ratio were calculated. For comparison, age- and sex-matched 30 children negative for severe acute respiratory syndrome coronavirus 2 were included in the study. Results. In the period from April to September 2020, 30 laboratory-confirmed cases of coronavirus-19, aged 0 – 17 years, were admitted to the Institute for Child and Youth Health Care of Vojvodina. The age was not a risk factor for the development of coronavirus-19 (p = 0.707; OR: 1.018; 95% CI: 0.927–1.119). The comparison of hematological parameters to assess the severe acute respiratory syndrome coronavirus 2 status showed that hemoglobin concentration (p < 0.01) and hematocrit (p < 0.01) were lower, and the percentage of neutrophil granulocytes (p < 0.05) was higher among severe acute respiratory syndrome coronavirus 2 positive children. Furthermore, it was found that some severe acute respiratory syndrome coronavirus 2 positive children had a higher (p < 0.01), while others had a lower (p < 0.01) percentage of lymphocytes. The systemic inflammatory index, the ratio of neutrophils to lymphocytes, and the ratio of platelets to lymphocytes, were not found to be statistically significantly different (p > 0.05). Conclusion. Low hemoglobin and hematocrit levels, a high percentage of neutrophil granulocytes, and a non-physiological percentage of lymphocytes (both, low and high) may have a diagnostic significance in children with severe acute respiratory syndrome coronavirus 2 infection.

Key words: Hematologic Tests; COVID-19; SARS-CoV-2; Coronavirus Infections; Child; Blood Cell Count; Hematocrit; Hemoglobins; Neutrophils; Lymphocytes

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Sažetak
Uvod. U poslednje vreme se javlja potreba za upotrebljenje lako dostupnih parametara za procenu SARS-CoV-2 infekcije kod dece. Materijal i metode. Sprovedena je retrospektivna studija u koju je uključeno 30 dece sa SARS-CoV-2 infekcijom, primljenih na Odeljenje za izolaciju Instituta za zdravstvenu zaštitu dece i omladine Vojvodine, od aprila do septembra 2020. godine. Pacijentima je određena kompletna krvna slika sa diferencijalnom krvnom slikom. Dodatno su izračunati sistemski inflamatorni indeks, odnos neutrofila i limfocita i odnos trombocita i limfocita. Za poređenje je u studiju uključeno još 30 dece negativne na SARS-CoV-2 sličnog uzrasta i pola. Rezultati. U periodu od aprila do septembra 2020. godine, 30 laboratorijski potvrđenih slučajeva COVID-19, uzrasta do 17 godina, primljeno je na Institut za zdravstvenu zaštitu dece i omladine Vojvodine. Uzrast nije bio faktor rizika za razvoj COVID-19 (p = 0,707; OR: 1,018; 95% CI: 0,927–1,119). Poredenje hematoloskih parametara prema SARS-CoV-2 statusu je pokazalo da su vrednosti hemoglobin (p < 0,01) i hematokrit (p < 0,01) bile niže, a procenat neutrofilnih granulo
cita (p < 0,05) viši kod dece pozitivne na SARS-CoV-2. Utvrđeno je da su pojedini SARS-CoV-2 pozitivni pacijenti imali viši (p < 0,01), dok su drugi imali niži (p < 0,01) procenat limfocita. Sistemski inflamatorni indeks, odnos neutrofila i limfocita i odnos trombocita i limfocita nisu se statistički značajno razlikovali (p > 0,05). Zaključak. Niske vrednosti hemoglobin i hematokrit, visok procenat neutrofilnih granulo
cita i neizolovanih limfocita mogu biti dijagnostički značajni kod dece sa SARS-CoV-2 infekcijom. Ključne reči: hematolski testovi; COVID-19; SARS-CoV-2; korona
virus infekcija; dece; kompletna krvna slika; hematokrit; hemoglobin; neutrofili; limfociti

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**Abbreviations**

SARS-CoV-2 – severe acute respiratory syndrome coronavirus 2  
WHO – World Health Organization  
COVID-19 – coronavirus-19 disease  
Ig – immunoglobulin  
CoVs – coronaviruses  
RNA – ribonucleic acid  
SARS – severe acute respiratory syndrome  
MERS – Middle East respiratory syndrome  
PCR – polymerase chain reaction  
SII – systemic inflammatory index  
NLR – neutrophil-lymphocyte ratio  
PLR – platelet-lymphocyte ratio

**Introduction**

An emerging microbe, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was firstly confirmed in patients with pneumonia in Wuhan, China, in December 2019 [1]. It spread rapidly throughout the whole country and, eventually, to other parts of the world. The global pandemic was declared by the World Health Organization (WHO) on March 11th, 2020. [2] There have been more than 72 million confirmed cases of coronavirus so far, and the death toll exceeds one million, which indicates that this infection is a public health emergency [3]. Coronaviruses (CoVs) are a family of ribonucleic acid (RNA) viruses that replicate in the cytoplasm of infected cells and are prone to mutations, helping them to adapt and to infect the host anew [4]. The CoVs are predominantly responsible for respiratory diseases. So far, there have been two significant outbreaks - severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS) in 2002 and 2012, respectively [5]. The SARS-CoV-2 causes the coronavirus disease (COVID-19), a form of respiratory and systemic zoonosis, which presents with a wide range of symptoms. While some patients may suffer from serious disease forms, such as SARS, others have mild upper-respiratory-tract symptoms, or they are even asymptomatic. These asymptomatic patients especially contribute to the complexity of disease transmission [6]. Making timely diagnosis and therapeutic decisions is of the utmost importance. Given that at the time a significant number of patients with respiratory symptoms are flooding in and the results of etiological polymerase chain reaction (PCR) tests cannot offer results instantly, it is necessary to utilize other, easier accessible laboratory parameters as criteria for SARS-CoV-2 infection [7]. Currently, the official guidelines (Guidelines of the National Health Commission of China for COVID-19, 5th edition and the WHO interim guidelines) recommend white blood cell count and number of lymphocytes as significant for early diagnosis [8]. Several alterations of hematological parameters have been described in the current literature. It has been noted that in the early stage of the disease onset, total leukocyte count is normal and the lymphocyte count is reduced. The lymphocyte count is found to be significant for the prognosis of patients with COVID-19. Another common laboratory finding is thrombocytosis [9]. Some also suggest that eosinopenia is an important predictor of the outcome [8]. All data were primarily documented from adult cases. The information about hematological abnormalities in children with SARS-CoV-2 infection are very limited worldwide, since the incidence of the disease in the pediatric population is significantly lower than in the adults [9]. Additionally, the interpretation of these results varies to a significant extent [10]. Our study aimed to analyze hematological parameters alterations in children with SARS-CoV-2 infection. The secondary objective of the study was to compare the baseline hematological parameters in SARS-CoV-2 positive children with age- and sex-matched SARS-CoV-2 negative patients.

**Material and Methods**

A single-center retrospective study included 30 SARS-CoV-2 pediatric patients of both sexes. Patients with COVID-19 admitted to the Isolation Department of the Institute for Child and Youth Health Care of Vojvodina from April to September 2020 were enrolled in the study. The diagnosis of COVID-19 was confirmed by real-time PCR performed on nasopharyngeal and throat swab specimens. The demographic data and the hematological findings from the blood samples collected on the day of admission were recorded. None of the patients received any kind of treatment prior to blood sampling. The samples were tested using a hematology analyzer Siemens Advia 2120 for complete blood count with differential white blood cell count. The following hematological tests were collected: white blood cells count, red blood cells count, hemoglobin concentration, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, red blood cell distribution width, platelet count, platelet distribution width, plateletcrit, mean platelet volume, absolute neutrophil count, neutrophil percentage, absolute lymphocyte count, lymphocyte percentage, absolute monocyte count, monocyte percentage, absolute eosinophil count, eosinophil percentage, absolute basophil count, and basophil percentage. Furthermore, systemic inflammatory index (SII), neutrophil-lymphocyte ratio (NLR), and platelet-lymphocyte ratio (PLR) were calculated. For comparative analysis, age- and sex-matched 30 SARS-CoV-2 negative patients were also enrolled in the study. All of them were children with different non-infectious and non-inflammatory diagnosis admitted to the Institute for Child and Youth Health Care of Vojvodina and tested with an immunochromatographic immunoglobulin (IgM/IgG) antibody assay (Innovita COVID-19 IgM/IgG Rapid Test) for COVID-19 prior to admission. Given that pediatric population is very heterogeneous regarding reference ranges, it was not possible to split them into age- and sex-matched subgroups, due to insufficient patient number in both groups. Therefore, hematological values were categorized according to the age- and sex-related hematology reference ranges: "physiological", "below physiological" and "above physiological". Informed consent
Table 1. Age and sex distribution in the control and the COVID-19 group

| Patient features/Karakteristike pacijenata | Total/Ukupno (n=60) | Control group/Kontrolna grupa (n=30) | COVID-19 group/COVID-19 grupa (n=30) | p-value p-vrednost |
|--------------------------------------------|----------------------|---------------------------------------|--------------------------------------|-------------------|
| Gender/Pol                                 |                      |                                       |                                      |                   |
| Female/Ženski                              | 35 (58.3%)           | 17 (56.7%)                            | 18 (60%)                             | 0.793*            |
| Male/Muški                                 | 25 (41.7%)           | 13 (43.3%)                            | 12 (40%)                             |                   |
| Age (years)‡ Uzrast (godine)               |                      |                                       |                                      |                   |
| Female/Ženski                              | 5.93 ± 5.44          | 6.20 ± 5.63                           | 5.67 ± 5.32                          | 0.712‡            |
| Male/Muški                                 | 5.50 (0.30 – 10.00)  | 5.50 (0.25 – 10.00)                   | 5.00 (0.31 – 10.25)                  |                   |

Table 2. Comparison of hematological parameters between the control and COVID-19 group

| Hematološki parametar | Control group/Kontrolna grupa (n=30) | COVID-19 group/COVID-19 grupa (n=30) | p-value p-vrednost |
|-----------------------|---------------------------------------|--------------------------------------|-------------------|
| RBC                   | 96.7                                  | 0                                    | 3.3               |
| HGB                   | 100                                   | 0                                    | 76.7              |
| HCT                   | 100                                   | 0                                    | 60.0              |
| MCV                   | 76.7                                  | 13.3                                 | 10.0              |
| MCH                   | 80.0                                  | 10.0                                 | 90.0              |
| MCHC                  | 83.3                                  | 3.3                                  | 76.7              |
| RDW                   | 76.7                                  | 10.0                                 | 73.3              |
| WBC                   | 86.7                                  | 10.0                                 | 70.0              |
| Neutr#                | 86.7                                  | 3.3                                  | 63.0              |
| Lymph#                | 86.7                                  | 3.3                                  | 63.0              |
| Mono#                 | 83.3                                  | 10.0                                 | 6.7               |
| Eos#                  | 86.7                                  | 0                                    | 13.3              |
| Baso#                 | 93.3                                  | 6.7                                  | 0                 |
| Neutr%                | 93.3                                  | 6.7                                  | 0                 |
| Lymph%                | 93.3                                  | 6.7                                  | 59.3              |
| Mono%                 | 76.7                                  | 6.7                                  | 66.7              |
| Eos%                  | 83.3                                  | 0                                    | 16.7              |
| Baso%                 | 96.7                                  | 0                                    | 3.3               |
| PLT                   | 83.3                                  | 16.7                                 | 72.6              |
| MPV                   | 57.1                                  | 42.9                                 | 44.8              |
| PCT                   | 93.3                                  | 3.3                                  | 83.3              |

Legend/Legenda: RBC - Red Blood Cells/Eritrociti; HGB - Hemoglobin Concentration/Konzentracija hemoglobin; HCT - Hematocrit/Hematocrit; MCV - Mean Corpuscular Volume/Srednja zapremina eritrocita; MCH - Mean Corpuscular Hemoglobin/Srednja količina hemoglobina u eritrocita; MCHC - Mean Corpuscular Hemoglobin Concentration/Promsena koncentracija hemoglobin na litar eritrocita; RDW - Red Distribution Width/Raspodela eritrocita po volumenu; WBC - White Blood Cells/Leukociti; Neutr# - Absolute neutrophil count/Absolutna vrednost neutrofila; Lymph# - Absolute lymphocyte count/Absolutna vrednost limfocita; Mono# - Absolute monocyte count/Absolutna vrednost monocita; Eos# - Absolute eosinophil count/Absolutna vrednost eozinofila; Baso# - Absolute basophil count/Absolutna vrednost bazofila; Neutr% - Neutrophil percentage/Pecenat neutrofila; Lymph% - Lymphocyte percentage/Pecenat limfocita; Mono% - Monocyte percentage/Pecenat monocita; Eos% - Eosinophil percentage/Pecenat eozinofila; Baso% - Basophil percentage/Pecenat bazofila; PLT - Platelets/Trombociti; MPV - Mean Platelet Volume/Srednja zapremina trombocita; PCT - Plateletcrit/Platelekrit; PDW - Platelet Distribution Width/Raspodela trombocita po volumenu; Chi-square test i Fishers exact test/Chi-kvadrat test i Fishers test.
Comparison of hematological indices and ratios between the control and COVID-19 group

Table 3. Comparison of hematological indices and ratios between the control and COVID-19 group

| Index/Ratio | Control group (n=30) | COVID-19 group (n=30) | p-value/p-vrednost |
|-------------|----------------------|-----------------------|--------------------|
| SII‡ | 419.45 ± 264.06 | 1128.76 ± 1785.54 | 0.578 |
| NLR‡ | 1.53 ± 0.35 | 1.40 (0.95 – 1.97) | 0.662 |
| PLR‡ | 109.99 ± 44.46 | 169.63 ± 132.71 | 0.138 |

SII - Systemic inflammatory index/Sistemski inflamatorni indeks; NLR - Neutrophil-Lymphocyte ratio/Odnos neutrofila i limfocita; PLR - Platelet-Lymphocyte ratio/Odnos trombocita i limfocita; ‡ Mean ± standard deviation/median (interquartile range: Q1-Q3)/Aritmetička sredina ± standardna devijacija/medijana (interkvartalni opseg: Q1-Q3); † Independent samples t-test/Studentov t-test

was waived because of the retrospective nature of the study and the analysis was based on anonymous laboratory data. Statistical analysis (descriptive and inferential) was performed using the Statistical Package for the Social Sciences version 26.0 software. For categorical variables, Chi-square test and Fisher’s exact test were performed, for continuous variables with normal distribution t-test, and for non-normally distributed continuous variables Mann-Whitney U test were used. Before running any test for continuous variables, homogeneity of variance, i.e. normal distribution within each group, was checked. Binary logistic regression was used to determine risk factors for COVID-19. The study was approved by the Ethics Committee of the Institute for Child and Youth Health Care of Vojvodina.

Results

In total, 30 cases with SARS-CoV-2 infection were admitted to the Institute for Child and Youth Health Care of Vojvodina between April and September 2020, with an average age of 5.67 ± 5.32 years and a median age of 5.00 years; 60% of them were females, with a mean age of 5.27 ± 5.70 years and a median of 1.50 years, and 40% were males, with a mean age of 6.27 ± 4.89 years and a median of 6.50 years. In regard to the SARS-CoV-2 status, there was no statistically significant difference in gender and age (p > 0.05) (Table 1). Binary logistic regression analysis showed that age was not a risk factor for COVID-19 (p = 0.707; OR: 1.018; 95% CI: 0.927 – 1.119). No confounding factors were identified. A statistically significant difference was found between the test groups in hemoglobin concentration (p < 0.01) and hematocrit (p < 0.01) which were lower, while the percentage of neutrophils (p < 0.05) was higher among SARS-CoV-2 positive children. Furthermore, it was determined that some patients had a higher lymphocyte percentage (p < 0.01), while others had a lower lymphocyte percentage (p < 0.01) (Table 2). Comparison of hematological parameters according to SARS-CoV-2 status showed no statistically significant difference regarding white blood cell count, red blood cell count, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, red blood cell distribution width, platelet count, platelet distribution width, plateletcrit, mean platelet volume, absolute neutrophil count, absolute lymphocyte count, absolute monocyte count, monocyte percentage, absolute eosinophil count, eosinophil percentage, absolute basophil count, and basophils percentage (p > 0.05). The SII, NLR, PLR were not found to be significant (p > 0.05) (Table 3).

Discussion

Considering the global incidence and mortality risk of COVID-19, rapid and accurate diagnosis and therapeutic decisions are of utmost importance [11]. Real-time PCR tests provide definitive diagnosis of SARS-CoV-2 infection, but given that a lot of patients with respiratory symptoms are flooding in and laboratory capacities are limited and insufficient, the time it takes to get PCR tests results may be prolonged [12]. Consequently, there is a need to utilize other, easier accessible laboratory parameters as criteria for SARS-CoV-2 infection. The clinical hematology laboratory plays an important role by providing markers useful for quick assessment and triage of COVID-19 patients [1, 13]. In our study, 30 children with average age of 5.67 years and 40% males, tested positive for SARS-CoV-2 by real-time PCR. In a multicenter, multinational study conducted by Götzinger et al., the mean age was 5.0 years and 53% of patients were male [14]. Another study by Tagarro et al. showed a mean age of 3.3 years and 44% of the patients were male [15]. Furthermore, Cai et al. revealed that 40% of all patients were male and with a mean age of 6.17 years [16]. Thus, it can be said that our study is similar regarding the age-sex distribution in comparison to other researches in pediatric population. During the COVID-19 infection, patients are in hypermetabolic state and oxygen demands of peripheral tissues are increasing. Low hemoglobin concentration leads to decreased ability of patients’ body to support these increased demands [17]. Additionally, anemic children are two times more susceptible to lower respiratory tract infections [18]. In our study, low hemoglobin concentration and hematocrit val-
ues were found in SARS-CoV-2 positive children. The same result was noted in Lu et al. study [19]. Although neutrophilia is usually not present in viral infections, it can be found during the early phase of infection. However, it remains unclear whether it is the result of virus-induced cytopathy or host response to viral infection [20]. In our study group, the percentage of neutrophils was found to be higher in SARS-CoV-2 positive children. Even though neutrophilia has been commonly reported in adult population with COVID-19 [21], there is still not enough data to support this in pediatric population. Reactive lymphocytosis commonly occurs in patients with viral infections and it is more likely to begin in children than in adults [22], but in conditions like COVID-19, natural killer cells and T-cells become exhausted and their count starts decreasing which leads to lymphopenia [23]. Therefore, both increased and decreased lymphocyte count can be present in COVID-19 patients. We have determined that some patients had higher percentage of lymphocytes, while others had lower percentage of lymphocytes. The study of Xu et al. showed the same findings [24]. The SII, NLR and PLR are inflammation-related indicators [25, 26]. Eren et al. reported that low values of SII and NLR may have diagnostic properties in SARS-CoV-2 positive patients [26]. In our study, none of these indicators were found to be significant. This result is expected considering that children in our study group presented with mild to moderate symptoms.

Conclusion

Our results show that low hemoglobin/hematocrit, high percentage of neutrophils and non-physiological lymphocyte percentage (both, low and high) may have diagnostic properties in children with severe acute respiratory syndrome coronavirus 2 infection.

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