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Annals of Medical Research

journal page: www.annalsmedres.org

The importance of inflammation markers in the diagnosis of COVID-19 in children

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ARTICLE INFO

Keywords:
Children
COVID-19
NLR
PLR
Polymerase chain reaction

Received: Dec 02, 2021
Accepted: Jul 24, 2022
Available Online: 25.07.2022

DOI:
10.5455/annalsmedres.2021.12.643

Abstract

Aim: To predict the presence of COVID-19 by readily available hematological and biochemical tests.

Materials and Methods: A total of 58 children with signs indicative of COVID-19 (28 PCR positives for SARS-CoV2 and 30 negatives) were included in this retrospective study. Clinical and laboratory parameters have been compared between these groups.

Results: White blood cell counts, RDW, MPV, NLR, PLR, LDH and CRP values were found to be higher in pediatric patients with positive PCR tests than children with negative PCR tests ($p<0.05$). There was a significantly positive correlation between CRP and NLR ($r=.566$, $p<0.001$), CRP and PLR ($r=.462$, $p<0.001$).

Conclusion: CRP, NLR and PLR, which are frequently used as inflammation markers, can help predict PCR positivity for SARS-CoV2. These easy-to-use tests can guide diagnosis in countries where access to PCR tests is limited.

Introduction

Acute respiratory syndrome coronavirus-2 (SARS-CoV-2) transmitted between humans through droplets, continued to spread globally, and the disease was named COVID-19 and classified as a pandemic by the World Health Organization [1]. According to our current knowledge, COVID-19 symptoms are milder in children in comparison to the adult population. Typical symptoms of pediatric patients diagnosed with COVID-19 include fever, coughing, sore throat, and mild gastrointestinal symptoms. However, while both adults and children show typical symptoms at the beginning of the outbreak, new atypical symptoms continue to be seen as the outbreak rises. Among these symptoms, complaints such as fatigue, anorexia, myalgia, headache and diarrhea come to the forefront [2]. The standard method used to confirm diagnosis of COVID-19 is the reverse transcription polymerase chain reaction (RT-PCR) test. Thoracic CT which is used in adults as an adjunct for the diagnosis is avoided as much as possible in children because of radiation exposure risk. Thus, the importance of laboratory tests becomes crucial for the diagnosis. There are several studies on laboratory outcomes in pediatric COVID-19 cases in the literature [3]. At this point, the importance of readily accessible laboratory tests yielding faster results than the standard PCR tests becomes crucial for the provisional diagnosis. There are several studies on laboratory outcomes in predicting pediatric COVID-19 cases in the literature [4,5]. However, majority of these studies are case reports or series. To the best of our knowledge, there is not any controlled study in the current literature that compares laboratory values of pediatric patients tested positive versus negative by RT-PCR with symptoms of upper respiratory tract infection. Therefore, we aimed to evaluate laboratory parameters of children with positive and negative results for COVID-19. We think that these tests can guide the diagnosis of COVID-19, especially in children presenting signs of upper respiratory tract infection, due to the easy accessibility and quick results.

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Materials and Methods

Study population and design

In this retrospective study, 58 patients hospitalized between March 2020 and May 2020 in the pediatric service with a preliminary diagnosis of COVID-19 have been included. RT- PCR tests has been applied to all patients. There was a history of contact with suspected cases of COVID-19 in the family and relatives of the patients. Children with any chronic diseases like any immune deficiency, cardiac, renal and neoplastic diseases were excluded. Demographic data of the children such as age and gender, weight and height have been collected from the recording system and Quatelet equation [body weight in kg / √ (body surface area in m2)] was used to calculate the body mass indexes (BMI). Hematological variables of white blood cell (WBC), neutrophil and lymphocyte counts, hemoglobin (Hb) , hematocrit (Htc), mean corpuscular volume (MCV), red cell distribution width (RDW), platelet (PLT), mean platelet volume (MPV), platelecrit (PCT), platelet distribution width (PDW) were analyzed with the XN-1000-Hematology-Analyzer (Sysmex Corporation, Kobe, Japan). The neutrophil-lymphocyte and platelet- lymphocyte ratios (NLR and PLR, respectively) were calculated by dividing the absolute number of neutrophil and platelets to lymphocyte counts. In addition, d-dimer was measured through the CS-2500 Analyzer (Sysmex Corporation, Kobe, Japan). Biochemical parameters including glucose, aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea, creatinine, lactate dehydrogenase (LDH), C-reactive protein (CRP) were measured by the Automatic Biochemistry Analyzer Abbott Architect c8000 (Abbott Laboratories, Abbott Park, IL, US). Thereby, assessed data were compared between PCR positive and negative groups. The association between relevant parameters was further assessed in the PCR positive group. The study protocol was approved by the local committee for human studies (Bolu Abant Izzet Baysal University Clinical Research Ethics Committee. Date/No: 29.05.2020/178) and conducted in accordance with the Declaration of Helsinki. Informed consent form was obtained from the study participants.

Statistical analysis

The data has been processed through SPSS version 22.0 (SPSS IBM Inc., Chicago, IL, USA). Normality of the variables was tested via the Kolmogorov-Smirnov method. The variables between the groups were compared by the Independent Student t-test for normally distributed variables, and the Mann-Whitney U test for non-normally distributed variables. Continuous variables were expressed as mean and standard deviation, whereas categorical variables have been given as frequency and percentage. The association between parameters was examined with Pearson's correlation analysis. A p value$<$0.05 was considered as statistically significant.

Results

Among the enrolled and tested children, twenty-eight were positive and 30 were negative with RT-PCR test for COVID-19. Demographic features of the groups are shown in Table 1. No statistical difference was observed between the two groups as regards to demographic data (Table 1). No statistical difference was observed between the two groups in regard with age, gender, weight, height and body mass index (BMI). Duration of fever, coughing and hospitalization were significantly higher in PCR positive chil-

Table 1. Demographic, anthropometric and clinical characteristics of the two groups.

Parameters	Covid-19 (PCR positive) n 28 (Mean±SD)	Covid-19 (PCR negative) n 30 (Mean±SD)	p value
Age (years)	5.41± 4.9	5.74 ± 4.8	0.818
Gender (n)			
Male Female	15 13	16 14	0.539
Weight (kg)	21.2± 17.2	28.4± 18.2	0.126
Height (cm)	101.2± 38.1	118.3± 35.9	0.083
Body mass index (kg/m^2)	17.4 ±1.9	17.6± 2.5	0.818
Duration (days) of			
Fever	2.81± 2.11	1.10 ± 0.80	<0.001 *
Cough	2.03± 2.26	0.13± 0.51	<0.001 *
Hospitalisation	6.59± 2.83	3.03± 1.42	<0.001 *

All data are mean± standard deviation.

Table 2. Hematological variables in both groups.

Parameters	Covid-19 (PCR positive) n 28 (Mean±SD)	Covid-19 (PCR negative) n 30 (Mean±SD)	p value
WBC (x10^9/L)	10.2±5.2	6.8±2.5	0.004*
Neutrophils(x109/L)	6.28±4.6	3.04±1.8	0.002*
Lymphocytes(x10^9/L)	2.9±1.9	2.9±1.6	0.992
Hemoglobin(g/dL)	12.8±1.8	13.3±1.5	0.361
Hematocrit (%)	36.7 ±5.0	38.0±4.8	0.306
MCV(fl)	78.3±8.7	79.6±6.2	0.508
RDW(%)	17.5± 2.9	16.1±1.2	0.035*
PLT (x10^9/L)	285.71±73.53	285.26.7±87.3	8 0.983
MPV(fl)	7.7±1.4	7.0±0.7	0.046*
PCT(%)	0.2±0.1	0.2±0.1	0.395
PDW(%)	17.4±1.2	16.8±1.2	0.079
NLR(%)	3.3 ±3.3	1.3±0.1	0.006*
PLR(%)	146.8 ±63.7	108.5±36.6	0.015*

WBC white blood cell, NLR neutrophil to lymphocyte ratio, PLR platelet to lymphocyte ratio, SD standard deviation, MPV mean platelet volüme, PLT platelet count, RDW red cell distribution width, PCT Plateletcrit, MCV mean corpusculer volüme, MPV mean platelet volüme, RDW red cell distribution width.

Table 3. Comparison of relevant biochemical parameters in both groups.

Parameters	Covid-19 (PCR positive) n 28 (Mean±SD)	Covid-19 (PCR negative) n 30 (Mean±SD)	p value
CRP(mg/dl)	34.2± 39.8	2.7 ± 7.8	<0.001 *
LDH(U/L)	317.7± 72.0	258.2± 61.0	0.028*
D-dimer(mg/L)	0.7± 0.5	0.5± 0.5	0.355
AST(U/L)	39.3 ± 26.8	28.6± 10.5	0.065
ALT(U/L)	22.9± 17.5	17.1± 10.2	0.133
Urea(mg/dl)	19.9± 7.4	22.3± 8.3	0.241
Creatinine(mg/dl)	0.5± 0.1	0.5± 0.2	0.957
Glucose(mg/dl)	94.4± 14.1	93.9± 13.6	0.900

AST aspartataminotransferaz, ALT alanin aminotransferaz, LDH lactatdehydrogenase, CRP C-reactive protein.

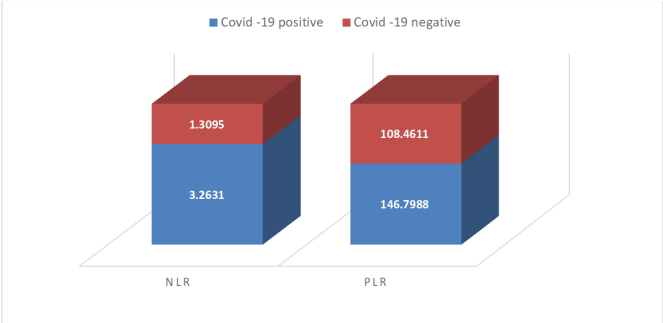

Figure 1. NLR and PLR values of Covid-19 positive and Covid-19 negative groups.

dren than the control group. One child in the PCR positive group showed respiratory distress for two days. Two of the PCR positive children exhibited skeletal symptoms of arthralgia and myalgia, and headache whereas none in the control group showed similar symptoms. Gastrointestinal symptoms such as diarrhea and nausea or vomiting were observed in a single child with PCR positive while these symptoms were experienced by three PCR negative children. Chest pain, hyposmia or anosmia were not detected in both groups.

Our study exposed a significant increase in mean WBC values in PCR-positive group than in PCR-negative children ($p<0.01$). On the other hand, neutrophil count was significantly higher in the PCR-positive group ($p<0.01$), while lymphocyte and thrombocyte counts did not differ among groups. Mean NLR as well as PLR were significantly higher in the PCR positive group compared to the control group ($p<0.01$ and $p<0.05$, respectively) (Figure 1).

We found significantly higher mean RDW and MPV values in the PCR-positive group than in the PCR-negative children ($p<0.05$ vs $p<0.05$, respectively). There were no statistically significant differences between the groups for other hematological variables ($p>0.05$) (Table 2). The comparison of biochemical parameters in two groups is shown in Table 3. There were higher mean CRP values as well as LDH values in the PCR positive group than in the control group ($p<0.001$ vs $p<0.05$, respectively).

The association between significant parameters was analyzed in the COVID-19 group. Accordingly, there was a significant positive correlation between CRP and NLR ($r=0.566$, $p<0.001$), and CRP and PLR ($r=0.462$, $p<0.001$).

Discussion

In this study, we observed that measured WBC, neutrophil, RDW, MPV, CRP and LDH levels were significantly higher as well as calculated NLR and PLR values in pediatric cases with COVID-19 than in the control group. However, the prognostic value of these parameters has not yet been clearly demonstrated in the literature.

With this study, we noticed a mean WBC count that was elevated to the upper limit of the normal and higher in the coronavirus-positive children than in the control group. In the face of significantly higher neutrophil counts in the PCR-positive group, we attributed the increase in leukocyte counts to elevated neutrophils. Cao et al. first declared a leukocyte count of 16.000/mm3 in a seven years old patient from Hubai state in China [6]. In an infant from the Middle East, Mansour et al. [7]. reported an elevated neutrophil count which was above normal limits. In studies, high neutrophil and leukocyte counts were found to be poor prognostic markers for the COVID-19 disease [8,9]. Therefore, we thought that simple measurement of neutrophil and leukocyte counts could be a prognostic biomarker recommended for the diagnosis and treatment of COVID-19.

The NLR value increase in relation to inflammation was found to be significantly higher in the patient group. In this regard, in a study conducted on 61 COVID-19 patients, the NLR value was reported as the most important predictive value that negatively affects the prognosis [10]. In another study by Yang et al., NLR was detected as a parameter with highest sensitivity to predict the severity of COVID-19 [11]. In the light of these previous studies, we thought that the significantly higher NLR in children with PCR positivity was associated with the severity of inflammation [12].

In our study, we preferred to investigate PLR, which is accepted as a new inflammation index. In the COVID-19 group, the PLR value was found to be statistically significant and high. In another study, it was thought that high PLR may be an inflammatory marker in the diagnosis and prognosis of the COVID-19 disease [13]. PLR, though its role has not been clarified definitely, has also been suggested as a prognostic parameter in COVID-19 [14,15]. Another platelet index MPV have been reported to signify a prognostic value in community-acquired pneumonia [16]. In our study, significantly higher MPV values were found in the PCR positive group. We thought that inflammation played a role in these results. Therefore, we determined that MPV values can be a guide in the diagnosis.

Mean RDW value was found significantly higher in COVID-19 group. Previously shown to be associated with

increased morbidity and mortality in a variety of diseases, RDW is a measure of variation in red blood cell volumes. With COVID-19, RDW also predicts the severity of the disease as well as other non-infectious diseases [17]. Particularly, RDW values higher than 14.5% have been shown to be correlated with increased mortality by a recent study conducted by Foy et al. in COVID-19 patients [18]. Though we did not observe increased mortality in our study group, our findings were compatible with formerly published studies.

Supportive to all other findings in our study, the most used parameter CRP evaluating the degree of inflammation for evaluation of infectious diseases has been found significantly higher in COVID-19 patients. CRP is of paramount index for diagnosing pulmonary infections and assessing its severity. CRP levels, independent of age, gender and physical factors, are associated with the degree of inflammation [19]. It has been reported that CRP levels could reflect the extent of lung lesions as well as the severity of the disease in the early stages of COVID-19 [20]. In a meta-analysis including 4662 patients, it was found that increased level of CRP was the most prevalent laboratory finding in COVID-19 patients. In the same study, CRP was reported to be above the normal range in 73.6% in COVID-19 patients [21]. Similar to the findings of our study, Munoz et al. [22]. and Mansour et al. [7]. both reported higher CRP levels in patients with COVID-19. Among other factors such as leukocyte levels, neutrophil counts, NLR, LPR, MPV and RDW, CRP showed the inflammatory state of during the children's coronavirus infections.

In our study, the mean LDH value in pediatric patients with COVID-19 was found to be significantly higher. There are limited studies reporting similarly elevated LDH levels in COVID-19 patients. Zhou et al. suggested LDH elevation as a diagnostic parameter for COVID-19. Apart from case series and studies, a single study of 136 children reported increased LDH levels in coronavirus infections [23]. This study has some limitations. First limitation of our study was its retrospective design. Second limitation is the small number of children included. Another potential limitation is the single environment in which the study has been carried out. This feature might be considered as a strength since all researchers implemented similar diagnostic or therapeutical procedures to the children studied. One of the strengths of the study is its controlled design. The latter, to our knowledge, is one of the first studies to propose such a simple and feasible hematological and biochemical test for the diagnosis of coronavirus infection. This may be advantageous for countries where diagnostic laboratory tests based on molecular and serological methods are not available.

Conclusion

Simple and easily applicable laboratory tests are of great importance, especially for developing countries, in the rapid diagnosis and treatment of COVID-19 in children. Inflammation-related CRP values, as well as NLR and PLR values could be a new predictive marker in pediatric COVID-19 patients.

Ethics approval

The study protocol was approved by the local committee for human studies (Bolu Abant Izzet Baysal University Clinical Research Ethics Committee. Date/No: 29.05.2020/178) and conducted in accordance with the Declaration of Helsinki.

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