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Research Article

The Importance of Lymphocyte Monocyte Ratio in SARS-CoV-2 Carriers

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Abstract

The aim was to determine hematological and biochemical changes in children who are SARS-CoV-2 carriers. The predictive factors of SARS-CoV-2 in children who are SARS-CoV-2 "super-spreaders" are the distinctive ratio between lymphocytes and monocytes (LMR) more than 2, with occasional eosinophilia, and a rapid, early, and strong increase of the serum LDH value. The predictive factors are useful for fast and cheap triage of children "from contact" until a PCR test result for SARS-CoV-2 arrives, and simultaneously, a pattern for the suspicion on the SARS-CoV-2 infection. The LMR is an adequate biomarker of immune response in SARS-CoV-2 carriers and it is different between various virus infections (SARS-CoV-2 vs. H1N1 influenza-like illness) in children. Our findings support a potential role for the LMR as a marker for adverse outcomes after asymptomatic SARS-CoV-2 in children.

Keywords: *Asymptomatic Infection Carrier; COVID-19; Pandemic; Child*

Introduction

The cruelty and severity of the coronavirus disease 2019 (covid19) pandemic admonished the human race that it has done overextension with changes, carried out on the Earth globe [1]. This is not the first pandemic of infectious disease, but the clinical presentation of coronavirus-19 (SARS-CoV-2) infection is significantly different from the clinical picture of all known viral infections and viral epidemics. The SARS-CoV-2 is specially adapted to avoid immune detection and suppress human immune responses [2]. During the covid19 pandemic, compared with adults, a small number of children had symptomatic disease, and the most often were asymptomatic (SARSCoV2 "super-spreader"), or a very small number of children had a severe clinical picture (respiratory and insufficiency of other organs) [3,4]. A neutrophil-lymphocyte ratio (NLR) and a lymphocyte-monocyte ratio (LMR) are important measures of systemic inflammation in children as they are readily available, cost-effective, could be calculated easily, have prognostic importance, and can be used in daily practice [5,6]. Based on the unusual clinical manifestation of SARS-CoV-2 infection, the questions arise: 1/ Are there hematological and biochemical changes in asymptomatic children with a positive PCR test for SARSCoV2 (SARSCoV2 carriers)? 2/ Would the NLR and LMR be predictor factors of asymptomatic SARSCoV2 infection carrier in children age (SARS-CoV-2 carriers)?

Aim of the Study

This manuscript aims are to analyze the basic hematological and biochemical parameters in children with a positive polymerase chain reaction (PCR) test for SARS-CoV-2 but without symptoms of this infection (SARS-CoV-2 carriers), and these findings to compare with reference values (r.v.) for the age of the child and that in influenza-like illness.

Patients and Methods

This is a study of 8 hospitalized patients because the confirmed covid-19 in their parents and it was in the time of beginnings of pandemic and when it has existed the fear of severe covid19 in children and when the covid19 in children was an unknown disease. The short time after that, we didn't hospitalize the SARSCoV2 carriers which justifies a small sample. Inclusion criteria were: 1) children up to 14 years of age, 2) laboratory-confirmed SARS-CoV-2 viral infection by PCR technique from the nasopharyngeal swab, on admission in the clinic, 3) asymptomatic patients on the day of admission in the clinic and during 14 days of hospitalization, 4) performed basic hematological, biochemical and radiographic diagnostics, on admission in the clinic. The diagnosis of the SARS-CoV-2 carriers was made based on the Guidelines for the Diagnosis and Treatment of Novel Coronavirus Pneumonia (6^{th} Trial Edition) proposed by the National Health Commission of the People's Republic of China [7]. The basic hematological values contained complete blood count parameters [8]. The basic biochemical values included transaminases (SGOT, SGPT) and lactate dehydrogenase (LDH), and as the basic inflammatory marker was determined a C-reactive protein (CRP) [8].

In all asymptomatic children, on admission to the clinic, the PCR test for SARS-CoV-2 was positive. After the 14 days of follow-up of asymptomatic children, the PCR test for SARS-CoV-2 became negative. From all subjects, we obtained data on a positive epidemiological history of SARS-CoV-2 virus infection, i.e. all subjects had one or more family members with positive PCR test for SARS-CoV-2. Observed signs and symptoms of SARS-CoV-2 infection were fever, cough, sore throat, shortness of breath, difficulty breathing, smell, taste dysfunction, sneezing, stuffy or runny nose, fatigue and tiredness, body aches, headache, diarrhea, vomiting, abdominal pain, conjunctival injection, watery eyes, mucous membrane changes, lymphadenopathy, and rash. The research was conducted by the Ethics Committee. A consent statement was not required for our paper because we used archive data.

Results

On the day of hospitalization, all subjects were in good general condition, with normal vital parameters, without the above-listed symptoms and all subjects underwent a complete blood count with leukocyte formula, CRP, transaminases, LDH, and X-ray of the lungs. Bearing in mind the small sample (n = 8), we didn't do statistical data processing, but only the presentation of the results individually per examinee, which is shown in table 1.

Patient	1	2	3	4	5	6	7	8
Gender (m-male, f-female)	f	m	m	m	f	f	m	f
Age (years + months/12)	2+9/12	8+6/12	5+3/12	0+8/12	1+8/12	13	1+9/12	2+10/12
Symptoms reported before PCR test and during 14 days of observation	No	No	No	No	No	No	No	No
Laboratory values								
Hematology								
Total white blood cell count (10^9/L) r.v. for age	4.22	5.70	4.70	17.50↑	7.70	4.50	12.66	10.12
	4.0-12.0	4.0-12.0	4.0-12.0	6.0-14.0	6.0-14.0	4.0-10.5	6.0-14.0	4.0-12.0

Neutrophils (%) r.v. for age	26.57	39.00	46.86	10.10	32.40	58.70	24.29	28.96
	54-62	54-62	54-62	54-62	54-62	54-62	54-62	54-62
Lymphocytes (%) r.v. for age	61.44↑	47.90↑	41.09↑	76.26↑	58.70↑	31.00	66.68↑	58.41↑
	25-33	25-33	25-33	25-33	25-33	25-33	25-33	25-33
Monocytes (%) r.v. for age	10.52↑	7.80↑	8.18↑	9.09↑	7.40↑	8.60↑	6.56↑	11.36↑
	3-7	3-7	3-7	3-7	3-7	3-7	3-7	3-7
Neutrophils/lymphocytes ratio (%) r.v. for age	0.4	0.8	1.1	0.1	0.6	1.9	0.4	0.5
	1.77 ± 1.71							
Lymphocytes/ monocytes ratio (%) r.v. for age	5.9	6.8	5.1	8.4	7.5	3.5	10.2	5.1
	2.85 ± 0.79							
Eosinophils (%) r.v. for age	1.15	5.10↑	3.06↑	4.39↑	1.30	1.60	2.20	0.97
	1-3	1-3	1-3	1-3	1-3	1-3	1-3	1-3
Basophils (%)	0.32	0.20	0.81	0.16	0.20	0.10	0.26	0.30
Hemoglobin (g/L) r.v. for age	116	140	126	114	113	126	118	112
	105-145	115-145	115-145	105-145	105-145	120-150	105-145	105-145
Erythrocyte indices								
MCV (fL)	76.9	81.0	81.8	71.3↓	71.8↓	84.4	72.7	74.8
r.v. for age	72-88	76-90	76-90	72-88	72-88	78-95	72-88	72-88
MCH (pg)	27.6	27.8	27.2	25.3	23.6↓	27.7	25.6	24.6
r.v. for age	24-30	25-31	25-31	24-30	24-30	26-32	24-30	24-30
MCHC (g/L)	359	343	332	354	328	328	352	328
r.v. for age	320-360	320-360	320-360	320-360	320-360	320-360	320-360	320-360
Platelet count (10^9/L) r.v. for age	204	184	304	361	257	241	363	460↑
	150-400	150-400	150-400	150-400	150-400	150-400	150-400	150-400
Inflammatory markers								
C-reactive protein (mg/L) r.v. for age	0.2	1.5	0.2	0.2	5.3	0.2	0.3	0.4
	0.8-7.9	0.6-7.9	0.6–7.9	0.8-11.2	0.8-7.9	0.6 – 8.1	0.8-11.2	0.8-7.9
Biochemistry								
AST, SGOT (U/l) r.v. for age	36	51	33	50	49	18	38	42
	20-60	15-50	15-50	22-63	20-60	10-40	20-60	20-60
ALT, SGPT (U/l) r.v. for age	12	61↑	14	22	17	11	18	14
	5-45	5-45	5-45	12-45	5-45	5-45	5-45	5-45
Lactate dehydrogenase (U/l) r.v. for age	634↑	611↑	585↑	788↑	657↑	375↑	653↑	570↑
	150-500	150-500	150-500	170-580	150-500	120-330	150-500	150-500
X-ray of the lungs	Normal	Normal	On both sides pronounced reticular pattern	Normal	Normal	On both sides pronounced reticular	Normal	Normal

Table 1: *Demographic, basic hematological and biochemical values, and radiological characteristics of asymptomatic patients with positive PCR for SARS-CoV-2.*

Instead of the control group, we used reference values of hematological and biochemical serum analyzes according to the age of the child [8]. The cut-off of NLR varies due to the different ages of children, what fore we used values of healthy children with a range of age-similar like in our study, and it is 1.77 ± 1.71 [5,6,9]. The reference value for LMR in children older than premature is approximatively 2.85 ± 0.79 [6,9]. CRP and cytological and biochemical urine findings were normal in all SARS-CoV-2 carriers. An X-ray with a pronounced reticular pattern was found in 2 of 8 patients (2/8). Platelet counts and transaminase values were within r.v. in 7/8 patients.

None of the subjects had any other comorbidities or other illnesses that had preceded to carrier state of SARS-CoV-2 infection.

Discussion

SARS-CoV2 is a single-stranded RNA virus from the coronavirus group with an unusual property of penetration in the cell and the ability to break down the beta-1 chain of hemoglobin so that the iron separates as free and hemoglobin loses its ability to bind oxygen, which leads to resistance hypoxemia and multi organic dysfunction named pediatric multisystem inflammatory disease, COVID-19 related [10,11]. Compensatory, the organism increases the synthesis of hemoglobin and ferritin, which explains their increased concentration in the serum, and it should be borne in mind that this compensation continues because too much hemoglobin has lost the ability to carry oxygen. High ferritin (non-toxic iron storage) is a bad prognostic sign [10,11]. During the early compensatory reaction of the organism, a larger number of monocytes are released from the bone marrow because they want to remove the excess iron from the organism. Differentiation and proliferation of the monocyte line are favored, what for developed a lymphopenia [5]. The lymphocytes are a regulator of the immune system, while the neutrophils are a marker of ongoing general inflammation due to releasing of some cytokines (tumor necrosis factor (TNF-α), interleukin 1 (IL-1), and IL-6) [12]. Thus, in SARS-CoV-2 carriers included in our serial case reports, lymphocytosis was found in 7 of 8 patients (7/8), with normal white blood cell (Le) count in 7/8 patients, monocytosis in 8/8 children, eosinophilia in 3/8 children, normal NLR in 8/8 children and normal LMR in only 1/8 children. Consequently, the LMR is high in 7/8 children. This shows that respiratory mucosal priming with cytokines occurred in SARS-CoV-2 carriers. It is difficult to find an adequate biomarker of immune response in SARS-CoV-2 carriers because there is a complex array of early immune-related mediators, but it would be LMR. The lymphocyte-monocyte ratio (LMR) would be a predictor factor of SARS-CoV-2 carriers in children, while the NLR hasn't this role. These findings indicate that SARS-CoV-2 carriers have, presumably, "silent" or "minimal" inflammation that induced monocytosis and change in LMR.

Besides this, we compared these findings with findings related to influenza-like illness. In those likely to have H1N1 virus infection exist relative lymphopenia with or without monocytosis [10], while in our SARS-CoV-2 carriers exist monocytosis with lymphocytosis and normal or increased leukocyte count predominate, what is the important differential diagnostic characteristic between these two infections. The LMR less than 2 with normal or low leukocytes is found in 90% of patients with influenza-like illness and has been suggested as a replacement instead of a rapid influenza test [10]. Unlike influenza carriers, SARS-CoV-2 carriers have an LMR greater than 2 with normal or increased leukocytes. Given that the covid19 pandemic is ongoing and there are indices that new waves will occur in the future, it is important to establish clear hematological differences between these two viral infections. For the suspicion of SARS-COV-2 infection and before the arrival of PCR results, it is useful to determine the relationship between lymphocytes and monocytes (LMR).

The rise of the hemoglobin (Hb), which was seen in symptomatic covid19 patients were not found in any asymptomatic patients. In most children (6/8) who are SARS-CoV-2 carriers, there were no changes in erythrocyte indices, which is an important difference with symptomatic covid19 patients. A fall of certain erythrocyte indices was found in a pair of children SARS-CoV-2 carriers, and that is a fall of the mean corpuscular Hb (MCH) in 1/8, and of mean corpuscular volume (MCV) in 2/8 SARS-CoV-2 carriers. Mean corpuscular Hb concentration (MCHC) was within normal limits in all SARS-CoV-2 carriers. Found values of erythrocyte indices and their mutual relations in a pair of patients (2/8) indicate the initial accelerated consumption of Hb and the change in the volume of erythrocytes into microcytic and hypochromic, in children who are carriers of SARS-CoV-2. Also, these findings indicate that SARS-CoV-2 carriers had, presumably, "silent" or "minimal" inflammation that can induce microcytic anemia.

The presented hematological changes were followed by multiple rises of lactate dehydrogenase (LDH) in serum in all SARS-CoV-2 carriers. The initial damage on cells (erythrocyte hemolysis) and respiratory tissue caused by SARS-CoV-2 are accompanied by the significant activity of this enzyme (LDH) in the blood [4,11,13]. Multiple increases of LDH level in serum were found in all (8/8) SARS-CoV-2 carriers, which means that the increase in LDH is an early biochemical sign of this infection in children. In all (8/8) children who were SARS-CoV-2 carriers, it was found multiple rises of serum LDH value with a normal red blood cell (Er) count, without an increase in Hb, already fall of erythrocyte indices, and the distinctive LMR, with occasional eosinophilia. Such hematological and biochemical findings may be a predictive factor of SARS-CoV-2 carriers in children. The SARS-CoV-2 carriers, in contrast to carriers of influenza virus, have other changes in leukocyte formula, and high LDH values [4,11,13]. Biochemical analysis of five specific serum LDH isoenzymes is a rapid method in contrast to the PCR test for SARS-CoV-2, the result of which is often waiting for many hours or almost a day, which suggests usability for quick triage of patients named the "contact from covid19 patients" or the "reservoir of SARS-CoV-2" [4,11,13].

Some countries, worldwide, have a frequent problem with a shortage of tests for diagnosis of SARS-CoV-2 (antigen or PCR-real-time), so our suggestions would be useful for fast and effective triage of asymptomatic patients and the implementation of stricter isolation for children of any age from 0 to 14 years, but it doesn't imply the replacement instead of a test titled PCR-real-time for SARS-CoV-2.

Conclusion

The children who are SARS-CoV-2 carriers stay at home and do not cough, haven't a fever, shortness of breath, difficulty breathing, or other characteristic symptoms and signs but there is a characteristic relationship between their basic hematological and biochemical changes. Note, the children who are SARS-CoV-2 carriers would be at a risk to develop post-covid19 later what is a reason more to identify carriers of SARS-CoV-2 promptly. The suspicion on the SARS-CoV-2 carriers in children's age may be based on the factors which imply hematological changes in the complete blood count (monocytosis, lymphocytosis, high LMR, eosinophilia, microcytic anemia) and a rapid, early, and strong increase of the serum LDH value. The practical applicability of the LMR higher than 2 for fast and cheap identification of the SARS-CoV-2 carriers in children needs to be considered in the future, as and for simple distinguishing from H1N1 influenza-like illness. The LMR is important for the following of the outcome of asymptomatic viral infection, and the spreading of SARS-CoV-2 infection and contact tracing in the context of COVID-19, and overall, it implicates the epidemiologic and clinical risks for subsequent sequels, while the NLR hasn't this role in SARS CoV2 "super-spreaders". Our findings support a potential role for the LMR as a marker for adverse outcomes after asymptomatic SARS-CoV-2. The LMR offers opportunities for research in children with post-covid-19 who were "SARS-CoV-2 carriers".

Supporting Information

S1 Table 1: Demographic, basic hematological and biochemical values, and radiological characteristics of asymptomatic patients with positive PCR for SARS-CoV-2. The numbers represent the means ± standard deviations.

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Contributorship

Drs Stojkovic and Dajic had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Drs Dajic and Kostic contributed equally as the co-first author.

Concept and design: Stojkovic, Dajic.

Acquisition, analysis, or interpretation of data: All authors.

Drafting of the manuscript: Stojkovic, Dajic, Kostic, Folic, Radovanovic, Medovic.

Critical revision of the manuscript for important intellectual content: All authors.

Administrative, technical, or material support: Dajic, Folic, Radovanovic, Medovic.

Supervision: All authors.

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