TOPICAL ISSUE

A.A. Parshina, Ye.V. Moskaleva, A.G. Petrova, L.V. Rychkova, O.B. Ogarkov, E.A. Orlova, A.S. Vanyarkina, E.A. Novikova, E.D. Kasantseva

SARS-CoV-2 VIRAL LOAD IN NEWBORNS WITH COVID-19

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The aim. Investigation of nasopharyngeal SARS-CoV-2 viral load in newborns with COVID-19 of different severity.

Materials and methods. The main group was composed of 44 newborns with RT-PCR confirmed COVID-19. 168 children aged from 1 month to 17 years old with RT-PCR confirmed COVID-19 were included in the group of comparison. SARS-CoV-2 viral load was measured as amount of viral RNA copies in 1 ml of nasopharyngeal mucosa using the regression model and presented as lg of the amount. The results presented as Me[Q1;Q3].

Results. SARS-CoV-2 viral load in newborns was detected significantly higher as compared to children aged 1-17 years: 3,2×106[5,7×104; 7,8×107] and 1,3×105[2,6×104; 1,2×107] respectively and no association has been revealed between nasopharyngeal SARS-CoV-2 viral load and disease severity, lung injury and the type of feeding.

Discussion. Nasopharyngeal SARS-CoV-2 viral load presumably reflects epidemiological circumstances and tends to decrease as the disease develops. It can be due to virus dissemination to lung tissue, vessel walls and other organs that followed by fall of number of viral particles in upper respiratory tract. While elimination of virus from mucosa on the early stages after infection probably depends on efficiency of innate immunity (which mechanisms can kill and/or impede virus invasion before immune response develops), viral load in blood and internal organs tissues, as well as favorable course of the disease, mainly depends on 'proportionate' immune response. So that, assessing the viral load and its significance for disease development should be performed considering the day after infection.

Conclusions. High level of nasopharyngeal SARS-CoV-2 viral load in newborns along with mostly mild COVID-19 course can be based on age-correlated features such as: immature immunity mechanisms, low expression of ACE2 receptors, the absence of comorbidity and intake of innate immunity factors while breastfeeding.

Keywords: newborns, COVID-19, viral load, SARS-CoV-2, children, new coronavirus infection.

PARSHINA Anastasiya A. – MD, PhD, Research Officer of the Laboratory of Infectology and Immune Prophylaxis in Pediatrics, Scientific Centre for Family Health and Human Reproduction Problems, e-mail: a.parshina.csma@yandex.ru. ORCID: 0000-0002-1458-2385; **MOSKALEVA Yekaterina V.** – MD, PhD, Research Officer of the Laboratory of Infectology and Immune Prophylaxis in Pediatrics, Scientific Centre for Family Health and Human Reproduction Problems, e-mail: mkatena@mail.ru. ORCID: 0000-0003-4196-0713; **PETROVA Alla G.** – MD, PhD, DSc, Chief Research Officer, the Head of the Laboratory of Infectology and Immune Prophylaxis in Pediatrics, Scientific Centre for Family Health and Human Reproduction Problems, E-mail: rudial75@gmail.com. ORCID: 0000-0002-7965-8061; **RYCHKOVA Lyubov V.** – MD, PhD, chief researcher, corresponding member of the Russian Academy of Sciences, Professor of the Russian Academy of Sciences, Director of the Scientific Centre for Family Health and Human Reproduction Problems, e-mail: iphr@sbamsr.irk.ru. ORCID: 0000-0002-0117-2563; **OGARKOV Oleg B.** – MD, Head of the Department of epidemiology and microbiology, Scientific Centre for Family Health and Human Reproduction Problems. E-mail: obogarkov@mail.ru. ORCID: 0000-0002-3168-1983; **ORLOVA Elizaveta A.** – MD, Research Assistant, Graduate Student of the Laboratory of epidemiologically and socially significant infections. E-mail: elizaveta.a.orlova@gmail.com. ORCID: 0000-0003-2169-0242; **VANYARKINA Anastasiya S.** – PhD, Research Officer of the Laboratory of Infectology and Immune Prophylaxis in Pediatrics, Scientific Centre for Family Health and Human Reproduction Problems, e-mail: avanyarkina@yahoo.com. ORCID: 0000-0001-8434-1600; **NOVIKOVA Evgenia A.** – MD, Research Assistant of the Laboratory of Infectology and Immunoprophylaxis in Pediatrics, Scientific Centre for Family Health and Human Reproduction Problems, e-mail: europe411@mail.ru ORCID: 0000-0002-9353-7928; **KASANTSEVA Ekaterina D.** – MD, Graduate Student of the Laboratory of Infectology and Immune Prophylaxis in Pediatrics, Scientific Centre for Family Health and Human Reproduction Problems, e-mail: kat.smile7@yandex.ru, ORCID: 0000-0003-0692-2295

Introduction. COVID-19 pandemic challenged worldwide healthcare system. Since understanding of the patterns of infection spread and mechanisms of its interaction with the human body are incomplete, it is of particular importance to search for reliable and accessible clinical and/or laboratory criteria, allowing to predict the features of the course and outcomes of the disease in a particular person.

The children's population is also susceptible to COVID-19 infection, but, unlike adults, it is more heterogeneous in the context of immune response characteristics. In general, the clinical experience accumulated during the fight against the new coronavirus infection suggests a milder course of COVID-19 in children: as acute respiratory viral infection and nasopharyngitis. The fact does not reduce the importance of identifying predictors of severe forms, such as pneumonia, acute respiratory distress syndrome, as well as multisystem inflammatory syndrome [3-5, 18, 27], which could contribute to the early diagnosis of the adverse development of the disease. As polymerase chain reaction (PCR) is generally accepted method for COVID-19 confirming, data on the amount of viral genetic material on the mucosa of the upper respiratory tract – nasopharyngial viral load (VL) can be used as an available prognostic criterion. In addition, this indicator, unlike the clinical picture, has not been studied in newborns, which makes the presented materials unique. The work continues a series of clinical and epidemiological studies of COVID-19 infection in the pediatric population [1, 2, 7-9, 15].

The aim. To study the level of SARS-CoV2 viral load in newborns with COVID-19 infection.

Material and methods. Two groups of children with a positive SARS-CoV-2 PCR test of a nasopharyngeal smear were examined. The main group included 44 newborns. The comparison group included 168 children aged from 1 month to 17 years, of which: 5 – children from 1 month to 1 year, 12 – children 1-3 years, 28 – children 3-6 years, 55 – children 7-11 years and 70 – children from 12 to 17 years. The nasopharyngeal VL of SARS-CoV-2 was determined in both groups. In children of the main group, the severity of the disease and objective signs of lung damage were additionally established, in accordance with the data of computed tomography (CT) of the chest or radiog-

raphy (Rg) of the lungs. The newborns were selected from children admitted to the “City Ivano-Matreninskaya Children's Clinical Hospital” Irkutsk, Russia in 2020-2021yy. with a diagnosis of a New Coronavirus Infection (COVID-19).

Baseline characteristics of the main group: boys predominate in the sexual structure – 65.9% (29/44); mean *(M)* age at admission is 4±3.1 days; weight and body length Me[Q1;Q3] at birth was 3210[2720;3600] grams and 51[49.5;54] cm, respectively, body weight at admission – 3300 [2780;3660] grams. The majority of newborns in the group were born full-term 88.6% (n=39), with a gestational age of 39[38.2;40] weeks, were immediately breastfed and 65.9% were breastfed during the treatment (n=29).

During inpatient treatment, the condition of 43.2% (n=19) newborns were assessed as mild, 38.6% (n=17) were of moderate, and 18% (n=8) were severe. CT- or Rg-signs of lung tissue injury ("ground glass", "cobblestone pavement", "reverse halo" symptoms) were identified in 54.5% (n=24). The most frequent clinical symptoms were fever 34±13.9% (n=15) and a runny nose of 29.5±13.4% (n=13). There were no cases of respiratory distress syndrome, multisystem inflammatory syndrome and deaths among the examined newborns.

The isolation of the genetic material of nucleic acids from the samples was performed with a set of reagents "RealBest extraction 100". RT-PCR studies were carried out using "Real-Best RNA SARS-CoV-2" reagents and with the Real-time CFX96 Touch (BioRad) amplifier according to the manufacturer protocol. The viral load (concentration of genome-equivalents of the SARS-CoV-2 virus in 1 ml of nasopharyngeal fluid) was determined according to the method described earlier [1].

Comparison between the groups was performed using Statistica 6.0 software (StatSoft, USA) using a nonparametric Mann-Whitney U-test, the differences were considered significant (*p) at $p<0.05$. Tabular data are presented in absolute numbers as median and interquartile interval – Me[Q1;Q3]. The data in the figures are presented as a decimal logarithm (lg) of the normalized number of copies of viral RNA in 1 ml of nasopharyngial mucus [1].

The study was approved by the Ethics Committee of the "Scientific Center for Family Health and Human Reproduction" (Protocol No. 6.1 of 06.19.2020). All participants or their legal representatives have signed an informed consent.

Results and discussion. The viral load of SARS-CoV-2 for all examined children was $1,3\times10^5$[$2,6\times10^4$; $1,2\times10^7$]. In the group of newborns – 3.2×10^6/ml (Table 1). that was significantly higher than in the pediatric population as a whole (according to Orlova E.A. et al.) (Figure 1) [1].

There were no differences of VL in groups of newborns with varying degrees of severity of the new coronavirus infection (Table 2).

Among children of all age groups, SARS-CoV-2 VL was maximum in the group of newborns; this indicator was significantly lower in children 3-11 years old (Figure 2).

The level of SARS-CoV-2 VL in breastfed newborns (n=23; $1,6\times10^6$[$5,8\times10^5$; $7,9\times10^7$]) did not differ (p=0.7) from VL in formula fed children (n=9; 1.8×10^7[7.7×10^5; $6,6\times10^8$]) (Table 4).

Additionally, a comparison of the LV level in newborns with CT or Rg signs of lung tissue injury and without them was performed (Table 3). There was no connection between the level of VL and the presence of lung injury (p=0.3) (Figure 3).

The accumulated clinical experience indicates a relatively low incidence of severe and complicated variants of COVID-19 in newborns. An asymptomatic or mild course does not exclude the fact of infection of children with the SARS-CoV-2 virus, which is confirmed by measuring the nasopharyngeal viral load, which is significantly higher than in the pediatric population as a whole [1, 6].

Today in the scientific literature, controversial data can be found concerning the relationship of the VL level with the severity of the inflammatory process. A number of studies reveal a positive correlation, however, most of the them indicate the absence of a clear linkage between VL and the severity of the disease,

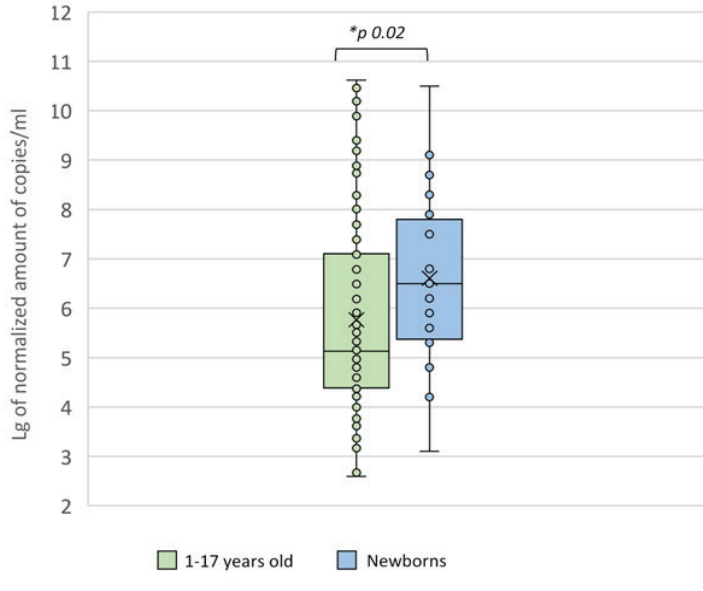

Fig. 1. SARS-CoV-2 viral load in 1-7 years old children and in newborns

Table 1

SARS-CoV-2 viral load in newborns

Me [Q1;Q3] copies/ml	Min-Max
3.2×10^6 [5.7×10^4; 7.8×10^7]	1.2×10^3 – 3.3×10^{10}

Table 2

SARS-CoV-2 viral load in newborns with COVID-19 of different severity

Disease severity	Viral load Me[Q1;Q3], copies/ml	Significance
Mild, n=19	2.4×10^6 [4.8×10^5; 2.5×10^8]	
Moderate, n=17	3.4×10^7 [1.5×10^7; 7.9×10^7]	$p^1=0.9$
Severe, n=8	2.2×10^5 [1.8×10^4; 3.2×10^6]	$p^1=0.2$; $p^2=0.2$

p^1 – as compared to Mild group (Mann-Whitney U-test), p^2 – comparison between Moderate and Severe group (Mann-Whitney U-test); the differences were considered significant at $p<0,05$.

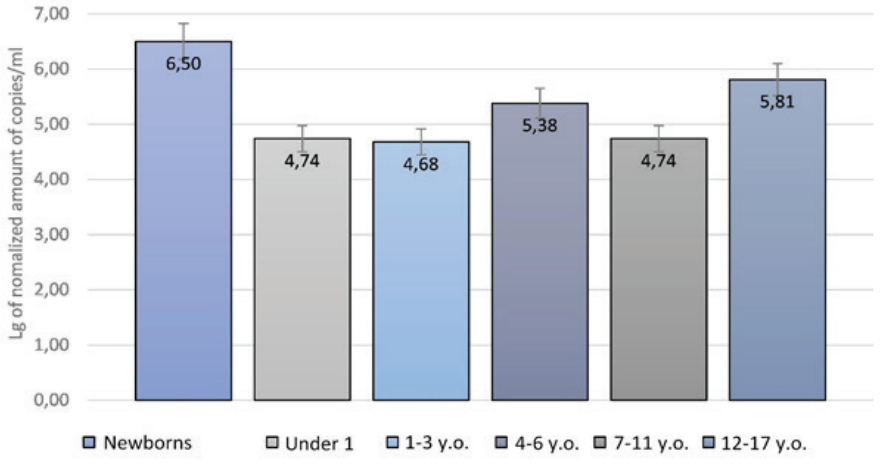

Fig. 2. SARS-CoV-2 viral load in children of different age

Table 3

SARS-CoV-2 viral load in newborns with and without lung injury

Group of newborns	Viral load Me [Q1;Q3] copies/ml	Min-Max
Lung injury, n=19	3.2×10^6 [7.6×10^5; 4.9×10^8]	3.2×10^5 – 1.3×10^9
No lung injury, n=9	1.5×10^6 [1.8×10^4; 3.4×10^7]	1.2×10^3 – 3.3×10^{10}

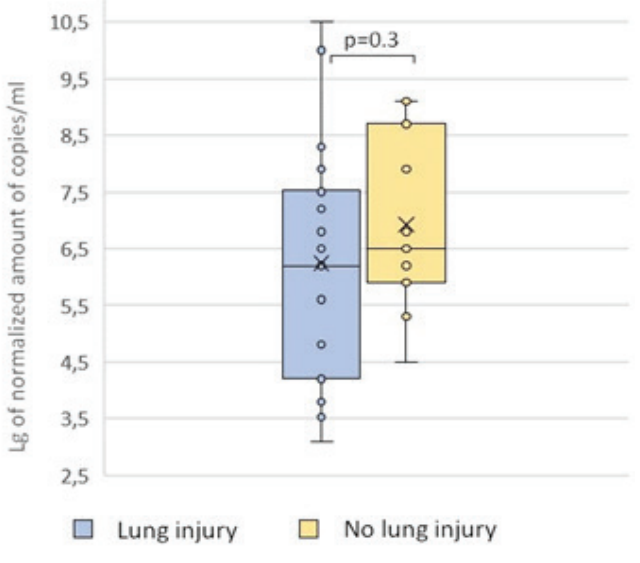

Fig. 3. SARS-CoV-2 viral load in newborns with lung injury and without lung injury

Table 4

SARS-CoV-2 viral load according to newborns`s type of feeding

Type of feeding	Viral load Me[Q1;Q3], copies/ml	Significance
Breast feeding, n=23	1.6×10^6[5.8×10^5; 7.9×10^7]	
Formula feeding, n=9	1.8×10^7[7.7×10^5; 6.6×10^8]	p=0.7

Сравнение выполнено с использованием U-теста Манна-Уитни, отличия значимы при p<0,05.

which is consistent with the data obtained in this work [19-24].

The results obtained suggest the following: VL level tends to decrease with the development of the disease, both in the case of positive dynamics and recovery, and in the case of an unfavorable course. After infection, viral particles accumulate in large quantities on the mucous membrane of the nasopharynx – the entrance gate of infection, therefore, asymptomatic and patients in the early stages of the disease are the most dangerous epidemiologically. Over time, the virus invades the cells of the body, replicate and disseminate into various tissues, which leads to a decrease in the number of viral particles on the mucous membrane of the upper respiratory tract, but an increase in VL in target organs (for example, in lung tissue or in the vessel wall), thus, inducing damage of specific systems [11, 12].

At the same time, the severity and outcome of the disease are determined not by the VL, but by the characteristics of individual reactivity, immune, in particular (considering the influence of comorbidity) [16, 17]. It follows that the level of nasopharyngial VL is, predominantly, a reflection of the epidemiological situation and effectiveness of nonspecific resistance mechanisms of the mucosa, while the number of viral particles in the internal environment depends mainly on the effectiveness of the immune response. Also it can indicate insufficiently studied, genetically determined features of somatic and/or immune cells, for example, the receptor apparatus, which makes some individuals prone to severe course [2, 12, 23-26].

This assumption is confirmed by the results of a number of studies aimed at studying the immune response in COVID-19: in asymptomatic patients and in patients with mild infection, specific antibodies to SARS-CoV-2 antigens are not detected or are present in low levels, unlike patients with a pronounced clinical manifestation [12, 13, 15, 16]. The fact allows hypothesize about the elimination of the virus from the mucous membrane before the development of a complete immune response. The period of time – from infection to the collection of biomaterial, also matters, as significantly affects the interpretation of the results.

Conclusions. The SARS-CoV-2 VL in newborns is significantly higher than in the pediatric population as a whole. At the same time, there is no evidence of the correlation between clinical severity and nasopharyngeal VL. The predominantly mild course of COVID-19 in newborns

may be due to age-related features: immaturity of their own immune mechanisms, low expression and functional activity of angiotensin-converting enzyme 2 receptors, which is necessary for invasion into the target cell, the absence of comorbidity and the consumption of a number of resistance factors with breast milk, including secretory antibodies [5, 10, 14, 18, 28].

Conflict of interests. The authors declare no conflict of interests.

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