EPIDEMIOLOGY

ASSESSMENT OF INDICATORS OF SPECIFIC HUMORAL IMMUNE AGAINST COVID-19 IN CHILDREN DURING THE DISTRIBUTION OF A NEW CORONAVIRUS INFECTION IN THE IRKUTSK REGION (2020–2021)

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ABSTRACT

Background. *There are many aspects of the development of immunity to the SARS-CoV-2 virus, that remain poorly understood, like the influence of age-related characteristics on the intensity of immunity and the course of the disease. Studies of the state of immunity are widely used, mainly in the adults. But questions of the patho- and immunogenesis in children remain unsolved. Determining the nature of seroconversion of antibodies to SARS-CoV-2 in this age group is important information for serological monitoring for targeted immunoprophylaxis of the population and forecasting the epidemic situation in the region.*

The aim. *Evaluation of the dynamics of seroprevalence of specific antibodies to SARS-CoV-2 in children of the Irkutsk region during the pandemic of a new coronavirus infection.*

Materials and methods. *Study was conducted among the child population of the Irkutsk region in the period June 2020 – December 2021 as part of the Rospotrebnadzor project to assess population immunity to SARS-CoV-2 in the population of the Russian Federation. The content of antibodies to SARS-CoV-2 was determined by ELISA using native commercial test systems.*

Results. *Population immunity among the child population of the Irkutsk region was characterized by an upward trend from 7.8 % at stage 1 to 98.4 % at stage 6 of the study. IgG to SARS-CoV-2 remained in 72.8 % and formed in 25.6 % of previously seronegative children. Antibodies were detected in 66.1 % of cases and persisted for up to 10–15 months after COVID-19 infection. The proportion of asymptomatic forms of infection among seropositive volunteers was 69.5 %, which determines the high intensity of the latent epidemic process.*

Conclusion. *The level of seroprevalence was 98.4 %. The current results of serological monitoring serve as a scientific basis for adjusting the list and scope of management decisions on the organization of preventive anti-epidemic measures, including vaccination.*

Key words: *children, COVID-19, SARS-CoV-2, humoral immunity, specific antibodies*

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ОЦЕНКА ПОКАЗАТЕЛЕЙ СПЕЦИФИЧЕСКОГО ГУМОРАЛЬНОГО ИММУНИТЕТА ПРОТИВ COVID-19 У ДЕТЕЙ В ПЕРИОД РАСПРОСТРАНЕНИЯ НОВОЙ КОРОНАВИРУСНОЙ ИНФЕКЦИИ В ИРКУТСКОЙ ОБЛАСТИ (2020–2021 гг.)

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РЕЗЮМЕ

Актуальность. *В настоящее время многие аспекты развития иммунитета к вирусу SARS-CoV-2 остаются малоизученными, в том числе влияние возрастных особенностей на напряжённость иммунитета и течение заболевания – широкое распространение получили исследования состояния иммунитета преимущественно у взрослых, а вопросы пато- и иммуногенеза этой инфекции у детей остаются нераскрытыми. Определение характера сероконверсии антител к SARS-CoV-2 в данной возрастной группе является важной информацией при серологическом мониторинге для проведения таргетированной иммунопрофилактики населения и прогнозирования эпидемической ситуации в регионе.*

Цель. *Оценить динамику серопревалентности специфических антител к SARS-CoV-2 у детей Иркутской области в период пандемии новой коронавирусной инфекции.*

Материалы и методы. *В рамках проекта Роспотребнадзора по оценке популяционного иммунитета к SARS-CoV-2 у населения Российской Федерации проведены исследования среди детского населения Иркутской области в период с июня 2020 г. по декабрь 2021 г. Содержание антител к SARS-CoV-2 определяли методом ИФА, используя отечественные коммерческие тест-системы.*

Результаты. *Популяционный иммунитет среди детского населения Иркутской области характеризовался тенденцией к росту – от 7,8 % на 1-м этапе до 98,4 % на 6-м этапе исследования. IgG к SARS-CoV-2 сохранились у 72,8 % и сформировались у 25,6 % ранее серонегативных детей. После перенесённой инфекции COVID-19 антитела выявлялись в 66,1 % случаев и сохранялись до 10–15 мес. Доля бессимптомных форм инфекции среди серопозитивных волонтёров составила 69,5 %, что обусловливает высокую интенсивность скрыто протекающего эпидемического процесса.*

Заключение. *Уровень серопревалентности составил 98,4 %. Текущие результаты серологического мониторинга служат научной основой для корректировки перечня и объёмов управленческих решений по организации профилактических противоэпидемических мероприятий, включая вакцинацию.*

Ключевые слова: *дети, COVID-19, SARS-CoV-2, гуморальный иммунитет, специфические антитела*

Для цитирования: Брюхова Д.Д., Дубровина В.И., Киселёва Н.О., Пятидесятникова А.Б., Корытов К.М., Балахонов С.В. Оценка показателей специфического гуморального иммунитета против COVID-19 у детей в период распространения новой коронавирусной инфекции в Иркутской области (2020–2021 гг.). *Acta biomedica scientifica*. 2023; 8(1): 239-246. doi: 10.29413/ABS.2023-8.1.24

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INTRODUCTION

COVID-19 was first reported in China in November 2019. Since then, this outbreak has rapidly developed into a global health emergency [1]. By May 2020, more than 4 million people had become infected with SARS-CoV-2. By January 2022, there were more than 350 million people infected with COVID-19 worldwide [2]. The disease is registered in almost all countries of the world, regardless of the climatic zone and the level of social and economic development. A significant proportion of asymptomatic forms of the infection characterizes the high intensity of the latently evolving epidemic process [3, 4]. The ubiquity of COVID-19 contributes to its progression among children. According to the literature, children account for up to 10 % of people infected with SARS-CoV-2 worldwide and 6–7 % in the Russian Federation [5–7]. One of the reasons is considered to be a significant frequency of asymptomatic forms of the infection, which remains unknown, but there is no doubt that this is due to the peculiarities of the child's immune system. It has been established that children with mild and/or asymptomatic course of the disease are able to produce specific SARS-CoV-2 antibodies (Abs), exerting a certain influence on the age structure of seroprevalence and herd immunity in general [8]. By the age of 4, more than 75 % of children are known to develop an immune response not only to seasonal, but also pathogenic coronaviruses [9].

The nature of the immune response to SARS-CoV-2 among children with various clinical manifestations from asymptomatic to MIS-C compared to the more common respiratory manifestations of COVID-19 among adults is unclear. Milder symptoms of the disease among children are the reason for low medical aid appealability, which in turn can lead to a decrease in detected cases of SARS-CoV-2 among children and adolescents. In addition, this age group with mild symptoms or with an asymptomatic course may contribute to the prevalence of COVD-19 among the population [10]. Therefore, the study of the virus-specific antibody reaction to SARS-CoV-2 among children and adolescents is important both for the development, adaptation and improvement of measures to control the incidence of COVD-19, and to address the issue of specific prevention of the child population.

THE AIM OF THE STUDY

Evaluation of the dynamics of seroprevalence of specific antibodies to SARS-CoV-2 in children of the Irkutsk region during the pandemic of a new coronavirus infection.

MATERIALS AND METHODS

The study was carried out as a part of the Rospotrebnadzor project to assess population immunity to SARS-CoV-2 among the population of the Russian Federation, taking into account the protocol recommended by WHO [11]. The studies were carried out according to a unified methodology developed by Rospotrebnadzor with the participation of the Saint Petersburg Pasteur Institute [12]. The work with volunteers was carried out in accordance with the ethical principles of the Declaration of Helsinki of the World Medical Association. The study was approved by the local Ethics committee of the Institute (Minutes No. 3 dated June 1, 2020, Minutes No. 7 dated November 11, 2021). Legal representatives of all volunteers got acquainted with the purpose and methodology of the study and signed an informed consent to participate in it.

A total of 384 randomly selected volunteers took part in the study, who passed a preliminary questionnaire and had a negative result of a study for the SARS-CoV-2 RNA by PCR. The same volunteers participated in each stage. The exclusion criteria during the immunological examination were: refusal of the patient's responsible representative to participate in the study, symptoms of acute respiratory infection with fever at the time of examination, the presence of acute or exacerbated chronic diseases 2 weeks before the study, lack of preparation required for the study.

The study of humoral immunity among children was conducted in 15 administrative territories of the Irkutsk region: from June 6 to July 7, 2020 – the 1st stage (384 subjects), from September 16 to September 25, 2020 – the 2nd stage (264 subjects), from December 7 to December 18, 2020 – the 3rd stage (249 subjects), from March 8 to March 14, 2021 – the 4th stage (212 subjects), from August 30 to September 4, 2021 – the 5th stage (224 subjects) and from December 13 to December 16, 2021 – the 6th stage (250 subjects).

In terms of gender of the volunteers participated in the study, the ratio of boys and girls was 1:1. All volunteers were divided into three age groups: 1–6, 7–13 and 14–17 years old.

The content of SARS-CoV-2 antibodies was determined by ELISA using a reagent kit for the analysis of blood serum or plasma for the presence of specific immunoglobulins G to the SARS-CoV-2 nucleocapsid produced by the State Research Center for Applied Biotechnology and Microbiology (Obolensk), as well as (at stages 5 and 6) using reagent kits for enzyme immunoassay detection of immunoglobulins G to the S-protein of the coronavirus – SARS-CoV-2-IgG-ELISA-BEST (Vector-Best LLC, Novosibirsk), and to the SARS-CoV-2 nucleocapsid proteins – SARS-CoV-2-IgG-Vector (State Research Center of Virology and Biotechnology VECTOR) and N-CoV-2-IgG PS (Saint-Petersburg Pasteur Institute).

The results were recorded qualitatively and were considered positive when the positivity index (PI) ≥ 1.1 was exceeded, the calculation of which was carried out according to the formula:

$$PI = (Sample\ OD) / critical\ OD\ (cut\ off),$$

where *Sample OD* is the measured optical density, and *critical OD (cut off)* is calculated in accordance with the instructions for the test system. For N-CoV-2-IgG PS, the results were considered positive when the quantitative IgG content exceeded ≥ 100 μg/ml.

Statistical data processing was carried out using variational statistics methods in Excel (Microsoft Corp., USA) and Statistica 6.0 (StatSoft Inc., USA). The probability le-

vel $p < 0.05$ was used to assess the statistical significance of the differences in the compared indicators. The normality of data distribution was determined using Kolmogorov – Smirnov test and Shapiro – Wilk test. The presented samples did not meet the criteria of normal distribution, therefore, nonparametric statistical methods for dependent samples (Wilcoxon test) were used in the comparative analysis. Pearson's chi-square (χ^2) test was used in contingency tables when comparing the level of seroprevalence at different observation periods. The data were expressed as median (Me) and interquartile range (Q25 %–Q75 %). Graphical data processing was performed using Excel (Microsoft Corp., USA).

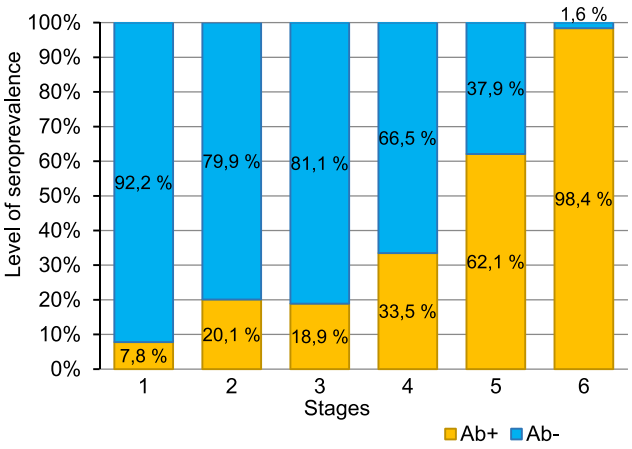

FIG. 1.
The level of seropositive volunteers among children at different stages of study

RESULTS AND DISCUSSION

Earlier, we showed that a low level of seroprevalence was formed in the Irkutsk region (stage 1 – 5.8 %; stage 2 – 12.1 %) at the onset of the epidemic outbreak and associated with a decrease in COVID-19 incidence. During the recrudescence period, seroprevalence reached 25.9 % (stage 3), which is 4.5 times more compared to stage 1 of the study [13]. It was found that herd immunity among the children of the Irkutsk region in the dynamics of observation tended to increase (Fig. 1) – from 7.8 % (stage 1) to 98.4 % (stage 6).

The number of seropositive volunteers was 30 out of 384 examined at stage 1, at stage 2 – 53 out of 264 subjects, at stage 3 – 47 out of 249 subjects, at stage 4 – 71 out of 212 subjects, at stage 5 – 139 out of 224 subjects, and at stage 6 – 246 out of 250 subjects (Fig. 1). Comparative analysis using χ^2 test showed statistically significant differences in the results at different stages of the study ($\chi^2 = 76.5$; df = 5; $p < 0.05$).

When comparing dependent samples of stages 1–4, Me ranged from 0.3 (0.2–0.5) at stage 1, 0.55 (0.3–0.9) at stage 2, 0.5 (0.3–0.8) at stage 3 and up to 0.7 (0.5–1.2) at stage 4 of the study (Fig. 2a). At stages 5 and 6, the quantitative test system N-CoV-2-IgG PS was used, so their comparison was carried out separately: Me was 140 (50–470) and 245 (40–610), respectively (Fig. 2b).

A high level of seroprevalence, which tended to increase, was established in Angarsk, Irkutsk, Usolye-Sibirskoye and Bratsk at all stages of the study. In December 2021, in some municipalities of the Irkutsk region (Angarsk, Taishet, Usolye-Sibirskoye), this parameter reached 100 % (Fig. 3). It is possible to estimate the reliability of statistical data in four localities (Bodaibo, Cheremkhovo, Bokhan, Ust-Ordynsky) only approximately, since the number of examined persons in these territories was less than 20 people.

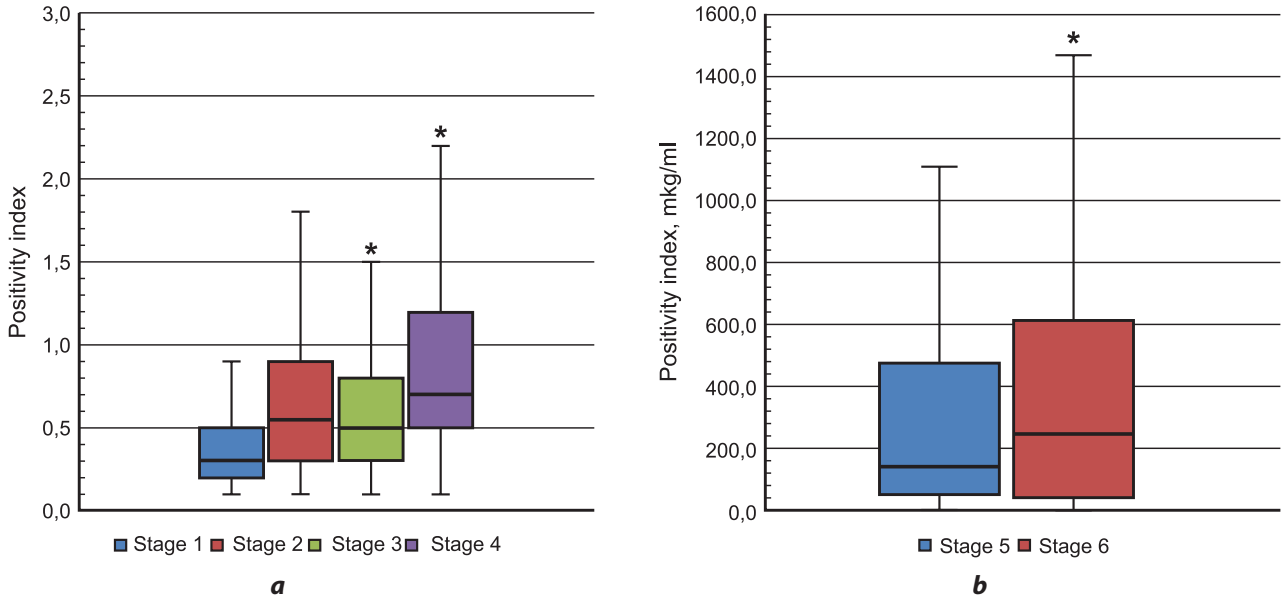

FIG. 2.
Positivity index values at each stage, Me (Q25 %–Q75 %): ***a*** *– stages 1–4, $p < 0.05$ compared to stage 1;* ***b*** *– stages 5 and 6, $p < 0.05$ compared to stage 5*

The maximum level of herd immunity among children at stages 1 and 2 of the study was noted in the group of 14–17 years (17 and 21 people respectively), at stages 3 and 4 – among preschool children (13 and 15 peo-

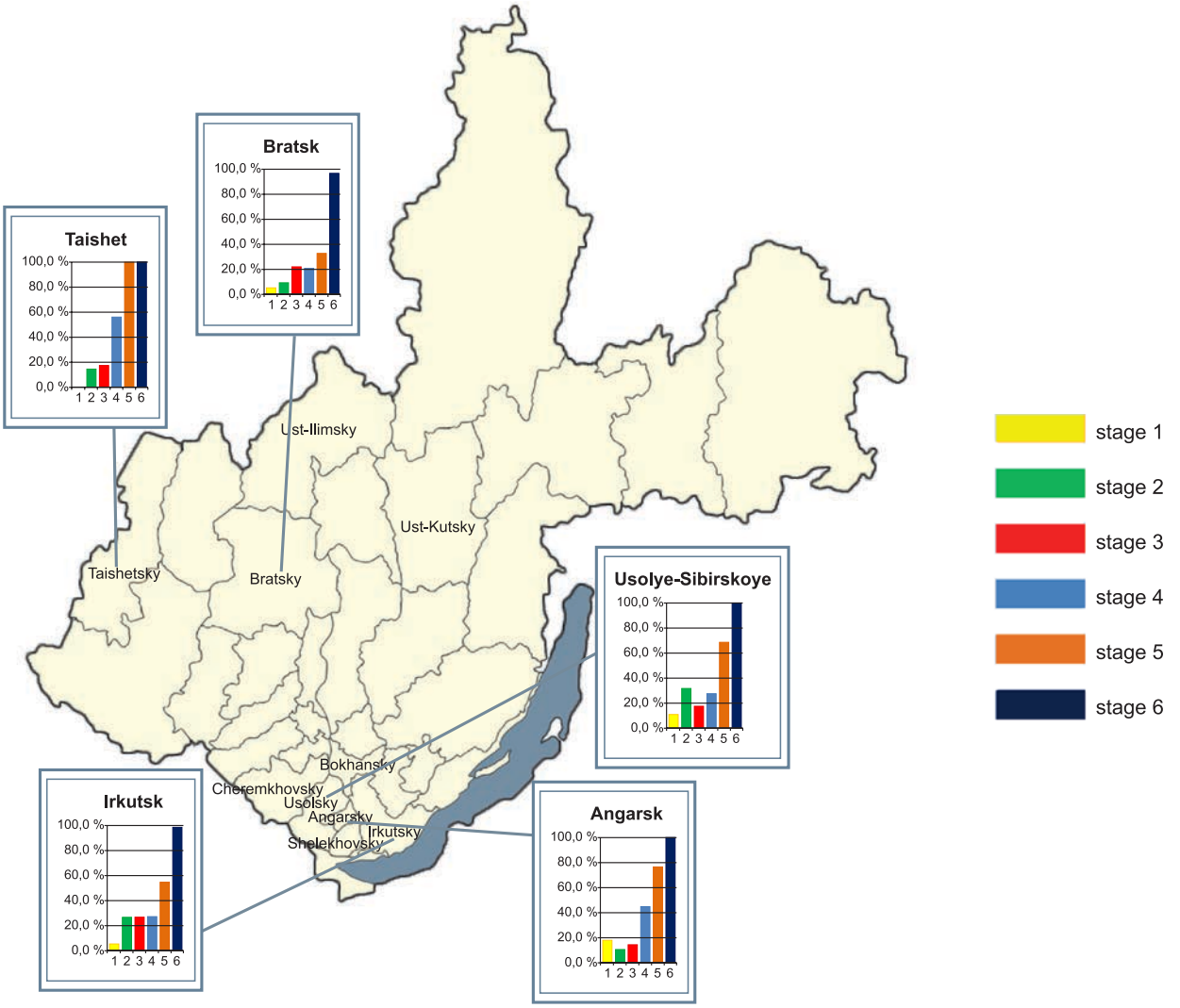

FIG. 3.

Indicators of seroprevalence among children in the territory of the Irkutsk region in the dynamics of observation

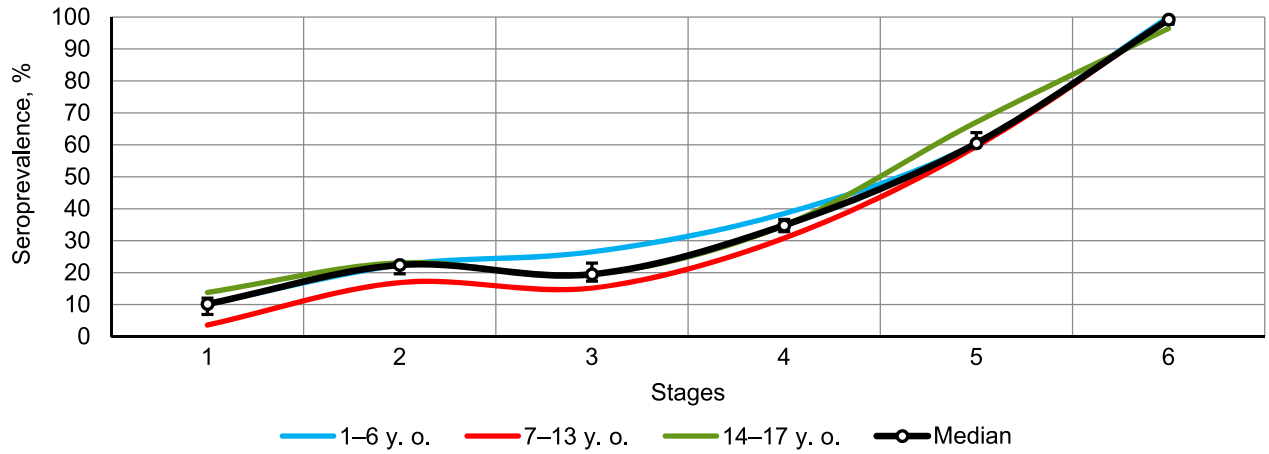

FIG. 4.

Dynamics of increasing seroprevalence to SARS-CoV-2 in children from stages 1 to 6. Colored lines – the process of changing the seroprevalence of children of the corresponding ages during the observation; the black bold line is the median; black vertical lines are the upper and lower quartiles (Q25 **%***–Q75 %)*

ple respectively), at stage 5 – in the group of 14–17 years (47 people), and at stage 6 – among preschoolers (38 people) (Fig. 4). During the study, the overall seroprevalence index among children increased by 12.6 times (from 7.8 to 98.4 %). 222 volunteers (52,9 %) with a primary negative result became seropositive. It is important to note that 57 seropositive (14.6 %) volunteers became seronegative 4–6 months after the detection of SARS-CoV-2 antibodies.

The percentage of children diagnosed with COVID-19 at stage 1 of the study was 0.52 % (2 people), at stage 2 – 1.5 % (4 people), at stage 3 – 7.6 % (19 people), at stage 4 – 7.1 % (15 people), at stage 5 – 12.1 %; (27 people) and at stage 6 – 15.2 % (38 people). At the same time, IgG to SARS-CoV-2 was detected in 50.0 % of volunteers at stage 1 of the study, at stage 2 – in 25 %, at stage 3 – in 42.1 %, at stage 4 – in 46.7 %, at stage 5 – in 81.5 %, and at stage 6 – in 100 %.

The analysis of seropositivity to SARS-CoV-2 at stage 6 of the study revealed a statistically significant preponderance of individuals with S-protein Abs (92.8 %) over the content of coronavirus nucleocapsid Abs (70.4 %). At the same time, the level of Abs to the studied antigens does not depend on age ($p > 0.05$). The study of the dynamics of antibody formation showed that Abs to Nc were detected on day 14 from the onset of the disease with a maximum increase in PI by day 185 and persisted up to day 432. Antibodies to SARS-CoV-2 S-protein were detected from day 7 after diagnosis and reached a peak by day 61, followed by a decrease to day 555.

Three possible types of seropositivity were identified among children based on the results of the study: twice positive (Nc+RBD), monopositive (Nc+ or RBD+) and negative (Nc– RBD–). The proportion of seronegatives was 1.6 %, the total proportion of RBD+ was 92.8 % and was statistically significantly higher ($p < 0.01$) than the Nc+ proportion – 70.4 %.

It is important to note that the total level of seroprevalence was 82.3 % during a comprehensive study of the herd immunity of the population of the Russian Federation (3,667 people), when calculating seropositivity to both antigens [14]. The results of a examination of persons with a history of COVID-19 (2020–2021) from the Irkutsk region indicate a long-term preservation of the humoral immune response among those who have had the infection. At the same time, the proportion of asymptomatic forms of infection among seropositive volunteers was 69.5 %, and in the Russian Federation – 76.9 %, which may indicate a high intensity of the latent epidemic process.

It was found that among the total cohort of examined volunteers aged 1–17 years, the number of children without a confirmed diagnosis of COVID-19, but having SARS-CoV-2 Abs, was 7.8 % at stage 1 (29 people), 20.1 % – at stage 2 (52 people), 18.9 % – at stage 3 (39 people), 33.5 % – at stage 4 (64 people), 62.1 % – at stage 5 (117 people) and 98.4 % – at stage 6 (208 people). No significant differences were found when assessing the correlation between the antibodies of volunteers with a confirmed diagnosis of COVID-19 and those who had this infection asymptomatically. A high proportion of seropositive children at stage 6 who had no manifestations of manifest infection (83.2 %) may indicate asymptomatic COVID-19 and the formation of full post-infectious immunity [14, 15], which may indicate a significant prevalence of undiagnosed cases of COVID-19 among children. Taking into account the proportion of cases of asymptomatic COVID-19, confirmed by the results of testing of seroprevalence of antibodies to Nc, the actual incidence of children is underestimated. In addition, children are less likely to have severe chronic pathology and are more likely to be infected by family members rather than vice versa, which may indicate a decrease in the virulence of the pathogen [15].

One of the most prominent manifestations of children's resistance to SARS-CoV-2 is the increased seroprevalence of Abs to pathogenic coronaviruses. An additional protective factor may be an increased level of seroprevalence of SARS-CoV-2 Abs, which is formed in response to the asymptomatic form of the course of coronavirus infection [14].

CONCLUSION

As part of the monitoring studies conducted for the first time among children aged 1–17 years, the fact of a widespread asymptomatic form of COVID-19 was confirmed, which does not exclude the formation of a humoral immune response. The proportion of seropositive to RBD and Nc was 92.8 and 70.4 %, respectively. Children have been found to be the main contributor to population immunity.

Antibodies were detected in 66.1 % of children with a PCR-confirmed diagnosis and persisted for up to 10–15 months to Nc and up to 15–18 months to RBD SARS-CoV-2 in the Irkutsk region after COVID-19 infection. Studies have shown that an increase in the level of Abs to RBD and Nc is accompanied by a decrease in the proportion of seroprevalence to SARS-CoV-2 antigens and levels the interage differences.

Thus, the results show a contribution of asymptomatic seroprevalent individuals to the level of humoral immunity to COVID-19 and a gradual decrease in the intensity of the epidemic of new coronavirus infection. The active formation of population immunity in combination with vaccination can serve as the main factor in ending the COVID-19 pandemic.

These results of serological monitoring can serve as a scientific basis for adjusting the list and volumes of epidemic control measures, making managerial decisions on the organization of preventive measures, including vaccination and forecasting the development of the epidemiological situation.

Conflict of interest

The authors of this article declare the absence of a conflict of interest.

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