Original article

UDC 616.98+578.834.1-06+616.3-008.1+616-053.2
https://doi.org/10.56871/CmN-W.2026.39.71.014

A model for predicting the absence of post-COVID gastrointestinal disorders in children who have had COVID-19 infection

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For citation: Polunina A.V., Novikova V.P., Bannova S.L., Dudurich V.V., Blinov A.E., Varlamova O.N., Dokhov M.A., Tikhomirova A.A., Dementiev N.A., Balashov A.L. A model for predicting the absence of post-COVID gastrointestinal disorders in children who have had COVID-19 infection. *Children's Medicine of the North-West*. 2026;14(1):169–178. (In Russ.). https://doi.org/10.56871/CmN-W.2026.39.71.014.

Received: 27.10.2025 **Revised: 26.01.2026** **Accepted: 20.03.2026**

ABSTRACT. ***Introduction.*** After recovery from COVID-19, many patients experience long-term sequelae known as post-COVID syndrome. The role of changes in the gut microbiota, particularly in relation to gastrointestinal symptoms following COVID-19, is of growing interest, although data on these changes in the post-COVID period remain controversial. ***Aim*** — to identify the main predictors of the absence of gastrointestinal symptoms in the post-COVID period in children using clinical, anamnestic, molecular biological, and immunological methods. ***Materials and methods.*** A database was created that included over 400 characteristics characterizing general information, clinical presentation, laboratory data, including procalcitonin and CRP levels, 16S microbiota sequencing, zonulin determination, stool PCR for SARS-CoV-2, and analysis of antibiotic and synbiotic prescriptions. The analysis was conducted in two groups of patients. Group 1 included 24 children who developed gastrointestinal symptoms one month after recovery, while Group 2 consisted of 23 patients who had no gastrointestinal symptoms. ***Results.*** Using a neural network, five features were identified that predict the absence of post-COVID gastrointestinal symptoms in children one month after recovery from novel coronavirus infection. The sensitivity of the neural network was 95.0%, specificity 87.5%, and accuracy 91.0%. ***Conclusion.*** The main predictors of the absence of gastrointestinal symptoms in the post-COVID period in children are the relative proportion of Actinobacteriota less than 0.24 in stool by 16S sequencing one month after recovery, the presence of Actinobacteria species less than 0.1 in stool by 16S sequencing one month after recovery, treatment with a synbiotic for 30 days, normal zonulin levels in stool, and the absence of a history of food allergies.

KEYWORDS: *COVID-19, children, post-covid period, functional gastrointestinal disorders, prognosis*

Funding. This study was not supported by any external sources of funding.

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Оригинальная статья

https://doi.org/10.56871/CmN-W.2026.39.71.014

Модель прогноза отсутствия постковидных гастроинтестинальных нарушений у детей, перенесших инфекцию COVID-19

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Для цитирования: Полунина А.В., Новикова В.П., Баннова С.Л., Дудурич В.В., Блинов А.Е., Варламова О.Н., Дохов М.А., Тихомирова А.А., Дементьев Н.А., Балашов А.Л. Модель прогноза отсутствия постковидных гастроинтестинальных нарушений у детей, перенесших инфекцию COVID-19. *Children's Medicine of the North-West*. 2026;14(1):169–178. https://doi.org/10.56871/CmN-W.2026.39.71.014.

Поступила: 27.10.2025 **Одобрена: 26.01.2026** **Принята к печати: 20.03.2026**

РЕЗЮМЕ. ***Введение.*** После выздоровления от COVID-19 у многих пациентов наблюдаются долгосрочные последствия, известные как постковидный синдром. Растущий интерес вызывает роль изменений микробиоты кишечника, особенно в связи с желудочно-кишечными симптомами после COVID-19, хотя данные об этих изменениях в постковидный период остаются противоречивыми. ***Цель*** — на основании клинико-анамнестических, молекулярно-биологических и иммунологических методов выявить основные предикторы отсутствия гастроинтестинальных симптомов в постковидный период у детей. ***Материалы и методы.*** Создана база данных, которая включала более 400 признаков, характеризующих общие сведения, клиническую картину, лабораторные данные, в том числе уровень прокальцитонина и С-реактивного белка, 16S секвенирование микробиоты, определение зонулина, ПЦР кала на вирус SARS-CoV-2, анализ назначения антибиотиков и синбиотика. Анализ проводился в двух группах пациентов. Группа 1 — 24 ребенка, у которых через месяц после выздоровления появились желудочно-кишечные симптомы, группа 2 состояла из 23 пациентов, не имевших никаких гастроэнтерологических симптомов. ***Результаты.*** С помощью нейронной сети выделены пять признаков, сопровождающих отсутствие постковидных гастроинтестинальных симптомов у детей через месяц после выздоровления от новой коронавирусной инфекции. Чувствительность нейронной сети составила 95,0%, специфичность — 87,5%, точность — 91,0%. ***Заключение.*** Основные предикторы отсутствия гастроинтестинальных симптомов в постковидный период у детей — это относительная доля *Actinobacteriota* менее 0,24 в стуле методом 16S секвенирования через месяц после выздоровления, наличие вида *Actinobacteria* менее 0,1 в стуле методом 16S секвенирования через месяц после выздоровления, лечение синбиотиком в течение 30 дней, нормальный уровень зонулина в стуле, отсутствие пищевой аллергии в анамнезе.

КЛЮЧЕВЫЕ СЛОВА: *COVID-19, дети, постковидный период, функциональные расстройства ЖКТ, прогноз*

Источник финансирования. Авторы заявляют об отсутствии внешнего финансирования при проведении исследования.

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ISSN 3034-6312 (Print) ISSN 3034-6320 (Online)

INTRODUCTION

Although the acute phase of the COVID-19 pandemic has ended, its long-term consequences remain a major healthcare concern [1]. At present, more than 1000 different genetic lines of the SARS-CoV-2 virus have been registered, possessing unique characteristics and different degrees of resistance to the immune response [2]. It has been established that even after recovery from various variants of COVID-19 infection, patients reveal signs and symptoms that persist after the disease or appear again, which is interpreted as post-COVID syndrome [3, 4]. Symptoms may be associated with chronic inflammation, autoimmune diseases, chronic endotheliopathy, microthrombosis or persistence of the virus [3]. Changes in the composition of the microbiota may also play a role in long COVID, however data on the intestinal microbiota in the post-COVID period are contradictory [5, 6]. Recently, researchers' attention has been attracted by the relationship between changes in the intestinal microbiota and the presence of gastrointestinal manifestations after COVID-19-infection [7–11]. The characteristics of gastrointestinal symptoms in children and adults after new coronavirus infection are presented in Table 1.

In the study of A.A. Karasev (2023) it is stated that the total frequency of disorders of intestinal motor function (diarrhea/constipation) varies from 3.6 to 48%, abdominal pain of any localization — from 9 to 32% [12].

In another domestic observation, 25% of children complained of abdominal pain after COVID-19 infection [4].

In the work of A.V. Naletov et al. (2021) a predominance of diarrhea and abdominal pain in 76% of the studied patients after infection was noted [13].

One of the variants of gastrointestinal tract (GIT) damage after coronavirus infection is irritable bowel syndrome (IBS) [14, 15]. Its frequency, studied in several studies, varied from 0.6 to 11.6% [14, 15]. Prognostic factors for the development of IBS in the post-COVID period are insufficiently studied. In one study it was shown that in patients with acute gastrointestinal symptoms during COVID-19 the probability of developing persistent gastrointestinal symptoms is four times higher than in patients without acute gastrointestinal symptoms [16]. In three other studies [17–19] the presence of gastrointestinal symptoms in the acute phase was named a precursor of IBS, whereas in other studies [20, 21] no correlation between gastroenterological symptoms during illness and symptoms in the post-COVID period was found. It is considered that the pathogenesis of IBS in the post-COVID period is mainly associated with dysfunction of the "gut–brain" axis [22, 23].

Currently, the role of prebiotics and probiotics in the restoration of normal intestinal microbiota

Table 1. Characteristics of gastrointestinal symptoms in children and adults after a new coronavirus infection (in absolute numbers)

Таблица 1. Характеристика гастроинтестинальных симптомов у детей и взрослых после перенесенной новой коронавирусной инфекции (в абс. числах)

Исследователи Researchers	Страна Country	Год Year	Количество больных Number of patients	Тошнота Nausea	Рвота Vomit	Диарея Diarrhea	Боли в животе Stomach ache
Obleagă et al. [7] (дети / children)	Румыния Romania	2023	21	21	4	21	21
D. Noviello et al. [8] (взрослые / adults)	Италия Italy	2021	164	–	–	–	16
J. Weng et al. [9] (взрослые / adults)	Китай China	2021	117	–	11	17	8
Huang et al. [10] (взрослые / adults)	Китай China	2021	1655	–	80	80	–
Anaya et al. [11] (взрослые / adults)	Колумбия Colombia		100	–	4	9	8

The table was compiled by the authors
Таблица составлена авторами

ISSN 3034-6312 (Print) ISSN 3034-6320 (Online)

and reduction of the severity of both gastrointestinal and respiratory symptoms in patients with COVID-19 is actively being studied [24, 25]. Data have been published on successful correction of dysbiosis in patients infected with COVID-19, which reveals the role of probiotics in the treatment of this new coronavirus infection [26]. The intake of certain prebiotics and probiotics may minimize gastrointestinal symptoms of COVID-19 and the consequences of antibiotic use, which aggravate these symptoms [27, 28]. However, factors contributing to the prevention of post-COVID gastrointestinal disorders in children have not been studied.

AIM

Based on clinical-anamnestic, molecular-biological and immunological methods, to identify the main predictors of absence of gastrointestinal symptoms in the post-COVID period in children.

MATERIALS AND METHODS

The calculation of the main predictors of the absence of gastrointestinal symptoms after coronavirus infection was based on the results of a clinical post-registration open observational prospective single-center study with minimal intervention. It was approved by the local ethics committee of the Federal State Budgetary Educational Institution of Higher Education "Saint Petersburg State Pediatric Medical University" of the Ministry of Health of the Russian Federation. Children with COVID-19 infection aged from 3 to 17 years were included in the study. The diagnosis of the new coronavirus infection and the severity of the disease were established according to methodological recommendations [29].

The children were examined in dynamics: at the beginning of the disease, after two weeks, upon resolution of the main symptoms of COVID-19 infection, and four weeks after recovery. Data collection was performed: complaints, medical history, clinical status, presence of complications, routine laboratory data and C-reactive protein (CRP) testing, 16S microbiota sequencing, stool zonulin testing, polymerase chain reaction (PCR) of stool for SARS-CoV-2 virus, analysis of prescribed medications, including antibacterial therapy and synbiotic (a synbiotic containing ≥1×10^9 CFU probiotic microorganisms, including *lactobacilli* — 6.6×10^8 CFU, *Lactobacillus acidophilus* LA-14 — 1.11×10^8 CFU, *Lactobacillus rhamnosus* GG — 1.11×10^8 CFU, *Lactobacillus casei* LC-11 — 1.11×10^8 CFU, *Lactobacillus paracasei* Lpc-37 — 1.11×10^8 CFU, *Lactobacillus plantarum* Lp-115 — 1.11×10^8 CFU, *Lactobacillus salivarius* Ls-33 — 1.11×10^8 CFU, including *bifidobacteria* — 3.3×10^8 CFU, *Bifidobacterium longum* Bl-05 — 1.11×10^8 CFU, *Bifidobacterium lactis* Bl-04 — 1.11×10^8 CFU, *Bifidobacterium bifidum* Bb-02/lactis — 1.11×10^8 CFU and the prebiotic component fructooligosaccharides — 0.538 g, hereinafter referred to as synbiotic at a dose of 1 capsule per day, treatment course — 30 days).

SARS-CoV-2 virus identification by PCR and 16S microbiota sequencing were performed in the laboratory "SERBALAB", Saint Petersburg (head — geneticist V.V. Dudurich). 16S DNA libraries were prepared in accordance with the Illumina protocol "16S Metagenomic Sequencing Library Preparation" (Part # 15044223 Rev. B). 5 ng of total DNA was used for amplification of the target fragment of the 16S rRNA gene using recommended primers for the V3 and V4 region. 25 PCR cycles were performed using KAPA HiFi HotStart ReadyMix (2X) (Roche Diagnostics, Switzerland). After purification of PCR products using SPRI beads, 5 ng of obtained amplicons were indexed using KAPA HiFi HotStart ReadyMix (2X) (Roche Diagnostics, Switzerland) and Nextera XT Index Kit (Illumina, USA). 8 cycles of index PCR were performed following the Illumina protocol. Libraries were sequenced on an Illumina MiSeq System. Stool zonulin testing was performed by enzyme-linked immunosorbent assay using the IDK Zonulin ELISA test system (Immundiagnostik AG, Germany). The mean value of the indicator declared by the manufacturer of the test system, according to examination of 40 practically healthy individuals, was 61±46 ng/ml. At values less than 83.15 ng/ml the result was considered normal, at 83.15–110.0 ng/ml — as increased concentration, more than 110 ng/ml — as high concentration.

The patient database included more than 400 features characterizing general information, clinical presentation, laboratory data, including procalcitonin and CRP levels, presence of symptoms, including gastrointestinal symptoms and their dynamics, disease severity, temperature response, presence of complications and comorbidities, microbiota composition by genus and species and diversity index, fecal zonulin level,

ISSN 3034-6312 (Print) ISSN 3034-6320 (Online)

presence of SARS-CoV-2 virus in stool, treatment with antibiotics and synbiotic. All variable features were assessed three times: at the beginning of the disease, two weeks after their resolution, and one month after recovery.

Analysis of all features was performed in two patient groups. The first group consisted of 24 children who developed symptoms such as abdominal pain and diarrhea one month after recovery. The duration of these symptoms within one month did not allow diagnosis of IBS, therefore we designated them as a group of children with post-COVID gastrointestinal symptoms. The second group consisted of 23 patients without any gastroenterological symptoms.

Checking the obtained data for compliance with a normal distribution law was performed using the Shapiro–Wilk test. If the data corresponded to the Gauss–Laplace distribution, typical values were presented as mean and standard deviation; if not corresponding – as median and first and third quartiles. Comparison of typical values between groups was performed using Student's t-test and Mann–Whitney test. The null hypothesis was rejected at a significance level of $p < 0.05$. A 95% confidence interval was calculated by Poisson distribution approximation via χ^2.

For determining predictors of absence of gastrointestinal symptoms after coronavirus infection using a neural network, a combined method of global optimization, random search, inertial and genetic algorithms was used.

RESULTS AND DISCUSSION

Using a neural network, five features accompanying the absence of post-COVID gastrointestinal symptoms in children one month after recovery from a novel coronavirus infection were identified. The data are presented in Table 2.

The sensitivity of individual variables in the neural network model was defined as the ratio of neural network errors without the factor feature of interest to the errors of the network with the factor feature of interest. The higher the sensitivity, the greater the importance of the studied feature, and the lower the prediction error of the neural network. The neural network sensitivity was 95.0%, specificity – 87.5%, accuracy – 91.0%.

Thus, the data obtained using the neural network indicate that the leading role in the development of gastrointestinal symptoms one month after recovery from a novel coronavirus infection is played by disturbances of the intestinal microbiota, namely the detection of the phylum *Actinobacteriota* and the species *Actinobacteria* in stool by 16S sequencing.

The results of the study are consistent with several studies in which significant groups of microor-

Table 2. Signs accompanying the absence of post-COVID gastrointestinal symptoms in children one month after recovery from novel coronavirus infection

Таблица 2. Признаки, сопровождающие отсутствие постковидных гастроинтестинальных симптомов у детей через месяц после выздоровления от новой коронавирусной инфекции

Признак **Sign**	**Чувствительность** **Sensitivity**	**Ранг** **Rank**
Относительная доля *Actinobacteriota* менее 0,24 в стуле методом 16S секвенирования через месяц после выздоровления Relative proportion of Actinobacteriota less than 0.24 in stool by 16S sequencing one month after recovery	6,8	1
Вид *Actinobacteria* менее 0,1 в стуле методом 16S секвенирования через месяц после выздоровления Actinobacteria species less than 0.1 in the stool by 16S sequencing one month after recovery	5,3	2
Лечение синбиотиком в течение 30 дней в возрастной дозе 1 капсула в сутки Treatment with a synbiotic for 30 days at an age-appropriate dosage of 1 capsule per day	3,6	3
Нормальный уровень зонулина в стуле Normal zonulin levels in the stool	2,3	4
Отсутствие пищевой аллергии в анамнезе No history of food allergies	1,8	5

The table was compiled by the authors

Таблица составлена авторами

ISSN 3034-6312 (Print) ISSN 3034-6320 (Online)

ganisms acting as predictors of disease severity in novel coronavirus infection were identified [30, 31]. In a study comparing the microbiome of patients with H1N1 virus and another group of patients with SARS-CoV-2 virus, five groups of microorganisms typical of the microbiota of COVID-19 patients were noted, including *Actinobacteriota*. The obtained data confirm the necessity of synbiotic treatment for the prevention of gastrointestinal disorders.

Recent studies have shown that food allergy in the medical history and increased intestinal permeability are pathogenetically related [32]. The influence of these features on the development of gastrointestinal disorders is confirmed by a number of publications dedicated to functional disorders of IBS [33, 34].

CONCLUSION

The main predictors of absence of gastrointestinal symptoms in the post-COVID period in children are: a relative proportion of *Actinobacteriota* less than 0.24 in stool by 16S sequencing one month after recovery, the presence of the species *Actinobacteria* less than 0.1 in stool by 16S sequencing one month after recovery, treatment with a synbiotic containing ≥1×10^9 CFU probiotic microorganisms, including *lactobacilli* — 6.6×10^8 CFU, *Lactobacillus acidophilus* LA-14 — 1.11×10^8 CFU, *Lactobacillus rhamnosus GG* — 1.11×10^8 CFU, *Lactobacillus casei* LC-11 — 1.11×10^8 CFU, *Lactobacillus paracasei* Lpc-37 — 1.11×10^8 CFU, *Lactobacillus plantarum* Lp-115 — 1.11×10^8 CFU, *Lactobacillus salivarius* Ls-33 — 1.11×10^8 CFU, including *bifidobacteria* — 3.3×10^8 CFU, *Bifidobacterium longum* Bl-05 — 1.11×10^8 CFU, *Bifidobacterium lactis* Bl-04 — 1.11×10^8 CFU, *Bifidobacterium bifidum* Bb-02/*lactis* — 1.11×10^8 CFU and the prebiotic component fructooligosaccharides — 0.538 g, for 30 days in age-appropriate dosage of 1 capsule per day, normal stool zonulin level, absence of food allergy in medical history.

ADDITIONAL INFORMATION

Authors' contribution. A.V. Polunina — article writing; V.P. Novikova — author of the concept and study design; S.L. Bannova — clinical data collection; V.V. Dudurich — genetic studies; A.E. Blinov — zonulin ELISA testing; O.N. Varlamova — biobank creation; M.A. Dokhov — predictive model calculation; A.A. Tikhomirova — article design; N.A. Dementyev — statistical database creation; A.L. Balashov — article editing and proofreading.

All authors read and approved the final version before publication.

Competing interests. The authors declare that they have no competing interests.

Consent for publication. Written consent was obtained from legal representatives of the patients for publication of relevant medical information within the manuscript.

ДОПОЛНИТЕЛЬНАЯ ИНФОРМАЦИЯ

Вклад авторов. А.В. Полунина — написание текста статьи; В.П. Новикова — автор идеи и составитель дизайна исследования; С.Л. Баннова — сбор клинических данных; В.В. Дудурич — выполнение генетических исследований; А.Е. Блинов — выполнение тестов ИФА на зонулин; О.Н. Варламова — создание биобанка материала; М.А. Дохов — расчет прогностической модели; А.А. Тихомирова — оформление статьи; Н.А. Дементьев — создание базы для статистической обработки; А.Л. Балашов — редактирование и вычитка статьи.

Все авторы прочли и одобрили финальную версию перед публикацией.

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

Информированное согласие на публикацию. Авторы получили письменное согласие законных представителей пациентов на публикацию медицинских данных.

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ISSN 3034-6312 (Print) ISSN 3034-6320 (Online)

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ISSN 3034-6312 (Print) ISSN 3034-6320 (Online)