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CYTOKINE–VITAMIN INTERACTIONS AS PREDICTORS OF COVID-19 SEVERITY IN CHILDREN: DEVELOPMENT AND VALIDATION OF A LOGISTIC MODEL

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Abstract.

While the majority of pediatric COVID-19 cases follow a mild clinical course, a distinct subset of patients develops severe disease associated with immune dysregulation and micronutrient deficiencies. Proinflammatory cytokines, including tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6), are pivotal in driving hyperinflammatory responses, whereas deficiencies in essential vitamins (A, D, B_6, B_9, B_{12}) may compromise antiviral defense mechanisms and immune homeostasis. Nevertheless, the combined prognostic utility of cytokine and micronutrient profiles in predicting disease severity among children remains insufficiently characterized.

Objective. *To develop and validate a multivariable logistic regression model for predicting severe COVID-19 in children integrating inflammatory biomarkers and micronutrient status.*

Materials and Methods. *Clinical and laboratory data were collected from 175 children with confirmed COVID-19. A subgroup of 112 children, for whom complete cytokine and micronutrient data were available, was selected for model development. Serum concentrations of TNF-α, IL-6, and vitamins B_9, A, and D were measured. Independent predictors were identified through stepwise logistic regression. Model performance was assessed in both training (n=89) and testing (n=23) cohorts using receiver operating characteristic (ROC) curve analysis and standard classification metrics. Ethical approval for this study was granted by the Bioethics Committee of I. Horbachevsky Ternopil National Medical University, Ministry of Health of Ukraine (Protocol No. 79, dated November 7, 2025). Written informed consent was obtained from the legal guardians of all participating children. Statistical analysis was conducted using Statistica 10.0 and SPSS 26.0 software. For continuous variables, 95% confidence intervals (95% CI) were calculated. The Kruskal–Wallis test (H-test) was employed to evaluate differences among the medians of multiple groups. Statistical significance was defined as a p-value less than 0.05. A multivariable logistic regression model with stepwise variable selection was utilized to develop the predictive tool. Diagnostic accuracy was evaluated through ROC curve analysis, including the area under the curve (AUC), sensitivity, and specificity. This study is part of the research project «Optimization of Diagnostic Approaches for Clinical and Pathogenetic Features of COVID-19 in Children with Comorbidities and Treatment Considerations» (State Registration No. 0123U100064, 2023-2025).*

Results. *Elevated serum concentrations of interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α), combined with deficiencies in vitamins B_9, A, and D, were identified as significant predictors of severe COVID-19 in children ($p < 0.05$). The developed model exhibited outstanding discriminatory capacity, achieving an area under the receiver operating characteristic curve (AUC) of 0.96, with a sensitivity of 87.5%, specificity of 93.3%, and overall accuracy of 91.3%. These metrics confirm the model's reliability in distinguishing between mild and severe disease courses.*

Conclusion. *The proposed logistic regression model represents a robust and clinically interpretable instrument for the early identification of children at risk of severe COVID-19. The integration of cytokine and micronutrient biomarkers into predictive algorithms may facilitate personalized interventions and enhance clinical decision-making in pediatric practice.*

Keywords: *COVID-19; Children; Disease Severity; Logistic Regression; Cytokines; Vitamins.*

Introduction

Although severe COVID-19 is less common in children than in adults, it remains a critical clinical concern due to the unpredictable progression of the disease in specific pediatric subgroups. Global epidemiological evidence suggests that 60-80% of infected children are either asymptomatic or exhibit only mild upper respiratory symptoms. However, 10-20% may develop pneumonia of varying severity, while a smaller yet clinically significant proportion progresses to severe or critical illness, necessitating hospitalization, respiratory support, or intensive care unit admission [1, 2]. This persistent risk emphasizes the critical need for early identification of children vulnerable to clinical deterioration.

Despite the lower mortality rates in pediatric populations compared to adults, sudden and rapid clinical deterioration has been documented even in previously healthy children, posing challenges for clinical management [3]. Among the proposed mechanisms, dysregulation of the host immune response has emerged as a key factor. The excessive and uncontrolled release of proinflammatory cytokines – termed a «cytokine storm»– has been strongly associated with the pathogenesis of severe COVID-19. In this context, interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) are consistently identified as biomarkers of systemic hyperinflammation [4, 5].

IL-6 is a pleiotropic cytokine produced by activated macrophages, dendritic cells, endothelial cells, and other immune cells. It mediates diverse biological effects, including the induction of acute-phase protein synthesis in the liver, promotion of B-cell differentiation, and modulation of T-cell responses [6].In COVID-19, elevated IL-6 levels have been linked to respiratory failure, the need for mechanical ventilation, and adverse outcomes, particularly in adult patients [7]. While pediatric studies remain limited, emerging evidence suggests that elevated IL-6 levels correlate with disease severity and may serve as an early indicator of complications in children [8].

Tumor necrosis factor-alpha (TNF-α) critically influences the regulation of inflammatory cascades, contributes to endothelial dysfunction, and facilitates

multi-organ involvement. Although most clinical evidence originates from adult populations, pediatric studies – particularly those examining multisystem inflammatory syndrome in children (MIS-C)—have consistently shown elevated TNF-α levels in severe cases. This cytokine may act synergistically with interleukin-6 (IL-6) to amplify hyperinflammatory responses [9, 10]. Consequently, TNF-α and IL-6 represent biologically plausible and clinically quantifiable indicators of disease severity in children.

In addition to cytokine dysregulation, accumulating evidence underscores the significance of nutritional status – specifically micronutrient levels – in modulating immune responses to SARS-CoV-2 infection [11]. Vitamin D has received particular attention in this context. Observational and case-control studies indicate that children with insufficient vitamin D levels are at higher risk of developing moderate or severe COVID-19 compared to those with adequate status [12, 13]. Vitamin D enhances antimicrobial activity in monocytes and macrophages by stimulating cathelicidin and defensin production, while simultaneously suppressing Th1 and Th17 responses and reducing the secretion of interleukin-2 (IL-2), IL-6, TNF-α, and interferon-γ [14]. By promoting an anti-inflammatory state, vitamin D may mitigate cytokine-induced lung injury [15].

Vitamin A also warrants consideration due to its essential role in maintaining epithelial barrier function, regulating mucosal immunity, and facilitating lymphocyte differentiation. Vitamin A also warrants consideration due to its essential role in maintaining epithelial barrier function, regulating mucosal immunity, and facilitating lymphocyte differentiation. Historical data from respiratory infections, including measles and respiratory syncytial virus (RSV), demonstrate that vitamin A deficiency increases morbidity and mortality, particularly in children under five years of age [16]. Recent studies suggest that insufficient vitamin A levels may similarly exacerbate COVID-19 outcomes, although pediatric-specific evidence remains limited [17]

B-group vitamins contribute to immune function through multiple mechanisms. Vitamin B_6 (pyridoxine) participates in amino acid metabolism and neurotransmitter synthesis while exerting anti-inflammatory effects by inhibiting nuclear factor kappa B (NF-κB) signaling and reducing cytokine production. Folate (vitamin B_9) is indispensable for DNA synthesis and repair, supporting lymphocyte proliferation; its deficiency has been associated with impaired antiviral responses and heightened inflammation. Vitamin B_{12} functions as a cofactor in one-carbon metabolism and redox balance, with observational studies linking low levels to poorer outcomes in viral infections [18]. Collectively, these findings suggest that a comprehensive micronutrient profile may complement cytokine biomarkers in risk stratification.

Recent clinical trials and meta-analyses have evaluated the efficacy of combined micronutrient supplementation in adults with COVID-19, revealing potential reductions in inflammatory markers and accelerated recovery [13, 16]. Nevertheless, robust pediatric data remain insufficient, particularly regarding the integrated prognostic value of immune and nutritional biomarkers. This evidence gap underscores the necessity for comprehensive approaches that concurrently assess cytokine activation and micronutrient deficiencies.

Objective

To develop and validate a multivariable logistic regression model for predicting the probability of severe COVID-19 in children, incorporating proinflammatory cytokine levels (interleukin-6, tumor necrosis factor-alpha) and micronutrient status indicators (vitamins A, D, B_6, B_9, and B_{12}).

Materials and Methods

A retrospective cohort study was conducted, encompassing 175 children aged 1 month to 18 years who were diagnosed with COVID-19 and received treatment between 2020 and 2024 at the Municipal Non-Profit Enterprise «Ternopil City Children's Clinical Hospital» in Ternopil, Ukraine. Diagnosis was confirmed through a positive polymerase chain reaction (PCR) test for SARS-CoV-2, using upper respiratory tract swabs (nasopharyngeal or oropharyngeal), and was supported by clinical manifestations and epidemiological history (contact with infected individuals or residence in high-prevalence regions), in accordance with the Ministry of Health of Ukraine Protocol No. 852, dated April 10, 2020. Children aged 1 month to 18 years with a confirmed positive PCR test for SARS-CoV-2, symptomatic COVID-19, and written informed consent from parents or legal guardians were eligible for participation

Serum levels of vitamins A, D, B_6, B_9, B_{12}, and homocysteine, as well as the proinflammatory cytokines TNF-α and IL-6, were measured in all patients using standardized enzyme-linked immunosorbent assay (ELISA) test systems. Analyses were performed on a Multiskan FC 357 immunoassay analyzer at the Interdepartmental Educational and Research Laboratory of I. Horbachevsky Ternopil National Medical University, Ministry of Health of Ukraine.

For predictive modeling, only children with complete cytokine and micronutrient datasets were included in the analysis. After excluding cases with missing biomarker data, 112 children remained for logistic regression modeling. Serum levels of TNF-α and IL-6 were quantified using ELISA, while vitamin A and 25-hydroxyvitamin D were measured via high-performance liquid chromatography (HPLC). Vitamins B_6, B_9 (folate), and B_{12} were assessed through chemiluminescent immunoassay.

COVID-19 severity was categorized according to World Health Organization pediatric criteria. Severe disease was defined by the presence of respiratory distress, oxygen saturation below 92%, radiographic evidence of pneumonia, or multisystem inflammatory syndrome in children (MIS-C).

The authors assume full responsibility for all aspects of this study, ensuring that any concerns regarding accuracy or integrity are thoroughly investigated and resolved. The study adhered to the principles of the Declaration of Helsinki (2013 revision). Ethical approval was obtained from the Bioethics Committee of I. Horbachevsky Ternopil National Medical University, Ministry of Health of Ukraine (Protocol No. 79, dated November 7, 2025). Written informed consent was secured from the parents or legal guardians of all participating children.

Data were analyzed using Statistica 10.0 and SPSS 26.0 software. A multivariate logistic regression model was constructed to develop the predictive tool, and diagnostic

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accuracy was assessed through receiver operating characteristic (ROC) analysis. Statistical significance was defined as a p-value less than 0.05.

A stepwise multivariate logistic regression approach was employed to identify independent predictors of severe COVID-19. The initial model incorporated TNF-α, IL-6, and serum concentrations of vitamins A, D, B_6, B_9, and B_{12}. Only variables demonstrating statistical significance ($p < 0.05$) were retained in the final model. Model training was performed using 80% of the dataset (n=89), with validation conducted on the remaining 20% (n=23).

Model performance was evaluated based on sensitivity, specificity, positive and negative predictive values, likelihood ratios, and overall accuracy. A ROC curve was generated, and the area under the curve (AUC) was calculated to determine predictive discrimination.

This study is part of the research project «Optimization of Diagnostic Approaches for Clinical and Pathogenetic Features of COVID-19 in Children with Comorbidities and Treatment Considerations» (State Registration No. 0123U100064, 2023-2025).

Results

The study included 175 children with confirmed COVID-19. The mean age was 5.95 ± 0.70 years (95% CI: 5.25-6.65); the cohort comprised 92 boys (52.6%) and 83 girls (47.4%). The largest proportion of cases occurred in the 1-5-year age group (34.9%), followed by the 5-15-year group (30.3%), children under 1 year (23.4%), and those aged ≥15 years (11.4%). Sex distribution differed significantly across age groups: boys predominated in the 1-5-year age group, while girls were more prevalent in the 5-15-year age group ($p < 0.05$).

Seasonal analysis revealed that 77.7% of infections occurred during autumn and winter, 20.0% in spring, and 2.3% in summer. Household contact was the primary source of infection (64.0%), while 2.3% of cases were attributed to school or daycare exposure. In 33.7% of cases, the source of infection remained unidentified.

Comorbid conditions were present in a subset of patients, including urinary tract infections (4.6%), neurological disorders such as cerebral palsy and epilepsy (4.0%), gastrointestinal and hepatobiliary diseases (4.0%), dysmetabolic nephropathy (2.9%), allergic history (2.9%), overweight (1.7%), congenital heart disease (1.7%), and bronchial asthma (0.6%). No significant association was detected between comorbidities and disease severity ($p > 0.05$).

Fever was the most common clinical manifestation, present in all enrolled children; high fever exceeding 38 °C was recorded in 64.6% of cases. Further respiratory symptoms included cough (36.0%), nasal congestion (28.6%), rhinorrhea (25.7%), sore throat (13.1%), and anosmia (6.9%), the latter predominantly reported in older children. General weakness was noted in 32.0% of patients, myalgia in 9.1%, and headache in 4.0%. Gastrointestinal involvement was likewise documented, comprising diarrhea in 15.4%, abdominal pain in 6.3%, vomiting in 4.0%, and nausea in 2.3%. Oxygen desaturation below 92% was identified in 14 children (8.0%), most frequently among adolescents aged 15 years or older. Radiological signs of pneumonia were detected in 21.1% of cases, confirmed by chest X-ray in 12.6%, lung ultrasonography in 7.4%, and chest computed tomography in 1.1%.

Based on clinical and radiological findings, 139 children (79.4%) were classified as having mild COVID-19, 24 (13.7%) a moderate course, and 12 (6.9%) severe disease. Severe cases were more frequently observed among adolescents, whereas mild disease predominated in younger age groups. Children with severe disease characteristically presented with high fever, pronounced weakness, nasal congestion, myalgia, gastrointestinal manifestations, and oxygen desaturation.

Table 1 presents the median values and interquartile ranges for vitamins A, D, B_6, B_9, and B_{12} in children with COVID-19 of varying severity, compared with the control group A consistent inverse trend is apparent: vitamin concentrations declined progressively with increasing disease severity Children with mild disease maintained values approximating those of the control group, whereas moderate and severe cases demonstrated marked reductions, most notably in vitamins A, D, and B_6. Vitamin B_{12} remained comparatively preserved, yet lower concentrations were nonetheless observed in severe disease. These findings indicate that monitoring and correction of vitamin deficiencies may play an important role in preventing progression to severe COVID-19 in paediatric patients (Table 1).

Table 1

Micronutrient levels and COVID-19 severity in children

Group	Vitamin A (ng/mL)	Vitamin D (ng/mL)	Vitamin B_6 (ng/mL)	Vitamin B_9 (ng/mL)	Vitamin B_{12} (pg/mL)
Control	487.00 (430.50-653.90)[a]	37.90 (24.60-43.25)[a]	57.56 (43.50-64.69)[a]	5.47 (4.76-7.03)[a]	461.10 (374.10-615.70)[a]
Mild	456.10 (394.00-566.00)[a]	30.91 (21.50-42.45)[ab]	56.80 (49.86-61.40)[a]	4.78 (3.36-6.36)[ab]	369.10 (270.20-484.10)[b]
Moderate	347.30 (279.60-503.10)[b]	29.10 (21.25-37.68)[b]	48.97 (35.60-57.67)[b]	4.22 (3.19-6.23)[b]	365.40 (288.50-442.40)[b]
Severe	242.90 (203.25-272.55)[c]	22.42 (18.99-30.50)[c]	39.41 (37.90-43.72)[c]	3.90 (2.27-5.10)[c]	310.90 (255.55-410.85)[c]
p-value	<0.001*	0.002*	0.015*	0.003*	0.001*

Note. *Values are presented as median (interquartile range, IQR). Overall between-group comparisons were performed with the Kruskal–Wallis test. Different superscript letters (a, b, c) within a column indicate statistically significant differences between groups (p < 0.05, Dunn's post hoc test).*

Table 2 presents the median values and interquartile ranges of key pro-inflammatory cytokines – IL-6 and TNF-α – in children with COVID-19 of varying severity, compared with the control group. The results reveal a consistent upward trend in cytokine concentrations corresponding to increasing disease severity. IL-6, a central marker of systemic inflammation, rose progressively from mild to severe cases, reaching peak values in children with severe COVID-19. TNF-α, another pivotal inflammatory mediator, demonstrated a pronounced elevation, with concentrations more than 50-fold higher in severe cases than in controls. These findings underscore the strong association between cytokine hyperactivation and disease progression in pediatric COVID-19, pointing to both prognostic and therapeutic relevance (Table 2).

Table 2

Cytokine levels and COVID-19 severity in children

Group	IL-6 (pg/mL)	TNF-α (pg/mL)
Control	5.7 (0.23-11.51)[a]	3.6 (0.12-8.1)[a]
Mild	80.36 (15.02-176.5)[b]	56.7 (12.76-119.1)[b]
Moderate	101 (32.04-274.1)[bc]	97.73 (80.55-178.5)[c]
Severe	151.90 (84.44-326.2)[c]	182.42 (172.42-205)[d]
p-value	<0.001*	<0.001*

Note. Values are presented as median (interquartile range, IQR). Overall comparisons between groups were performed using the Kruskal–Wallis test. Different superscript letters (a–d) within a column indicate statistically significant differences between groups based on Dunn's post-hoc test (p < 0.05). Groups sharing the same superscript letter do not differ significantly.

Multivariate logistic regression analysis was performed to identify the most significant predictors of severe COVID-19 outcomes in children. The model incorporated serum concentrations of vitamins A, D, B_6, B_9, B_{12}, alongside pro-inflammatory cytokines IL-6 and TNF-α as independent variables. This statistical method permits estimation of the probability of a binary outcome – in this case, severe versus non-severe COVID-19– from multiple continuous or categorical independent variables.

The model is structured on the foundation of the logistic function:

$$P = 1 / (1 + e^{-z})$$

Where:

- *P* is the predicted probability of severe COVID-19,
- *e* is Euler's number (approximately 2.72),
- *z* is the linear combination of predictors, calculated as:

$$z = a_0 + a_1x_1 + a_2x_2 + \ldots + a_nx_n,$$

where a_0 is the intercept, a_1–a_n are the regression coefficients, and x_1–x_n are the independent variables.

For the logistic regression analysis, a subset of 112 pediatric patients with available biomarker data (serum vitamins A, D, B_6, B_9, B_{12} and cytokines IL-6 and TNF-α) was included. The following seven biomarkers were initially considered as candidate predictors on the basis of their established pathophysiological relevance:

- tumor necrosis factor-alpha (TNF-α),
- interleukin-6 (IL-6),
- vitamin B_6,
- vitamin B_9 (folate),
- vitamin B_{12},
- vitamin A,
- vitamin D.

Backward stepwise selection identified five predictors with statistically significant associations with severe disease, which were retained in the final model: TNF-α, IL-6, vitamin B_9, vitamin A, and vitamin D. Vitamin B_6 and vitamin B_{12} were excluded on grounds of non- significance ($p > 0.05$).

The final logistic regression model was trained on 80% of the cohort (n = 89) and validated on the remaining 20% (n = 23). The estimated regression coefficients (B), standard errors (SE), p-values, and odds ratios (Exp(B)) for the predictors retained in the final model are presented in Table 3.

Table 3

Logistic Regression Coefficients for Predicting Severe COVID-19 in Children

Predictor	Coefficient (B)	Standard Error	p-value	Odds Ratio (Exp(B))
TNF-α	0.1413	0.0706	0.02	1.15
IL-6	0.0047	0.0023	0.002	1.00
Vitamin B_9	–0.0543	0.0271	<0.0001	0.95
Vitamin A	–0.0002	0.0001	0.002	1.00
Vitamin D	–0.2773	0.1387	0.02	0.76
Constant	–3.0153	0.8615	0.004	0.05

Note. Odds ratios (Exp(B)) are presented with two decimal places. p < 0.05 was considered statistically significant.

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The model demonstrated excellent explanatory power, with a Nagelkerke R^2 of 0.7905, indicating that approximately 79% of the variance in the outcome was accounted for by the included predictors.

The –2 log-likelihood of the final model was 86.00.

The final predictive equation used to calculate the log-odds (z) for each patient is as follows:

$$z = –3.0153 + 0.1413 \times \text{TNF-}\alpha + 0.0047 \times \text{IL-6} - 0.0543 \times \text{Vitamin } B_9 - 0.0002 \times \text{Vitamin A} – 0.2773 \times \text{Vitamin D}$$

Substitution of individual biomarker values into this equation yields the patient-level probability of severe disease via the logistic function.

The strength of association of each variable was reflected in its exponentiated coefficient (Exp(B)), which represents the odds ratio. Among all predictors, the strongest association with the probability of severe COVID-19 was attributed to vitamin A, followed by IL-6, vitamin D, vitamin B_9, and TNF-α.

These findings suggest that elevated pro-inflammatory cytokines and deficiencies in select vitamins contribute substantially to the risk of severe COVID-19 in children. The model thereby provides a robust, evidence-based tool for early risk stratification in pediatric clinical practice.

Model performance and predictive accuracy was evaluated on a separate test subset comprising 23 children (20% of the total study population). Of these, 15 children were clinically diagnosed with mild COVID-19 (Actual = 0), and eight with severe COVID-19 (Actual = 1), classified according to established clinical criteria and disease course.

Each child was assigned a predicted class based on the model's estimated probability. Of the 15 children with mild disease, 14 were correctly classified as non-severe (true negatives) and one was misclassified as severe (false positive). Of the eight children with severe COVID-19, seven were correctly identified (true positives) and one was incorrectly classified as non-severe (false negative). These results are summarised in the confusion matrix (Table 4).

Table 4

Confusion matrix and predictive performance of the logistic regression model (test set)

Indicators	Severe (Actual = 1)	Mild (Actual = 0)
Predicted = Severe (1)	7 (True Positive)	1 (False Positive)
Predicted = Mild (0)	1 (False Negative)	14 (True Negative)

Note. Positive predictive value (PPV): 87.5% (95% confidence interval [CI]: 52.9-97.8%); negative predictive value (NPV): 93.3% (95% CI: 70.2-98.8%).

To comprehensively assess the diagnostic performance of the proposed logistic regression model in predicting the severity of COVID-19 in pediatric patients, key evaluation metrics were derived from the confusion matrix.

Sensitivity, or the true positive rate, reflects the model's capacity to correctly identify patients with severe COVID-19. The model correctly classified 7 of 8 children with severe disease, yielding a sensitivity of 87.5%. This indicates that the majority of high-risk cases were detected and that false negatives were minimised.

Specificity, or the true negative rate, measures the proportion of children without severe COVID-19 who were correctly identified by the model. Of the 15 children with mild disease, 14 were accurately classified, yielding a specificity of 93.3%. This value reflects a strong capacity of the model to exclude severe disease in low-risk patients.

The positive predictive value (PPV) quantifies the probability that a child classified as high-risk actually has severe COVID-19. In this validation cohort, 7 of 8 children predicted to have severe disease were correctly classified, producing a PPV of 87.5%, confirming that a positive classification result is highly likely to reflect true severity.

The negative predictive value (NPV) represents the probability that a child predicted to be at low risk indeed has mild disease. Of 15 children classified as low-risk, 14 were true negatives, yielding an NPV of 93.3%. This result reinforces the model's reliability in excluding severe cases among those classified as low-risk.

Likelihood ratios were additionally calculated to quantify the model's discriminative ability. The positive likelihood ratio (LR+) – comparing the probability of a positive classification in children with severe disease to that in children without severe disease – was 13.06, indicating that a child with severe COVID-19 is approximately 13 times more likely to be classified as high-risk than a child with mild disease. The negative likelihood ratio (LR–) was 0.134, implying that a child with mild disease is approximately 7.5 times more likely to be classified as low-risk than a child with severe disease, which reflects strong model performance in minimising false negatives.

The overall accuracy of the model, defined as the proportion of correctly classified cases, was 91.3%, demonstrating that the logistic regression model provides highly reliable, clinically applicable predictions for distinguishing mild from severe COVID-19 in children.

Collectively, these performance metrics confirm excellent diagnostic accuracy and support reliable differentiation between children at high and low risk of severe COVID-19. The combination of high sensitivity and specificity with strong predictive values and likelihood ratios substantiates the model's potential utility for early clinical risk assessment.

Receiver operating characteristic (ROC) curve analysis was performed to evaluate the overall predictive performance of the model in identifying children at risk of severe COVID-19 (Figure 1). The ROC curve characterises the model's discriminatory ability across the full range of classification thresholds.

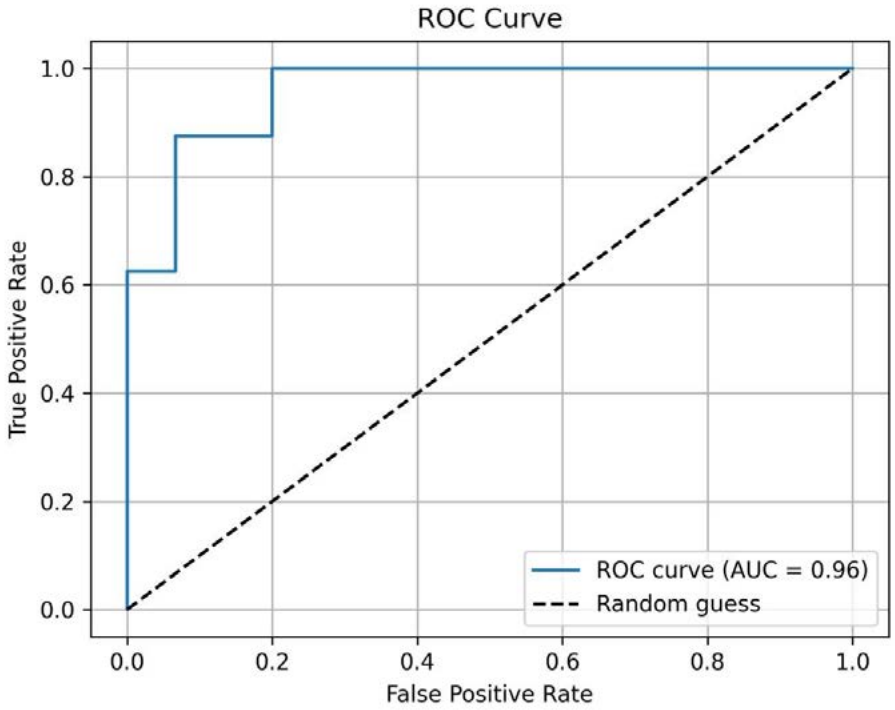

Figure 1. Receiver Operating Characteristic (ROC) curve for the logistic regression model predicting severe COVID-19 in children.

The area under the ROC curve (AUC) was 0.96, which, by accepted criteria, corresponds to outstanding discriminative ability (AUC 0.90-1.00). This result confirms that the model yields highly reliable predictions of severe disease in pediatric patients. The sensitivity and specificity derived from ROC analysis were consistent with previously reported values: 87.5% and 93.3%, respectively, with an overall accuracy of 91.3%.

These findings confirm the robustness and practical utility of the logistic regression model in clinical settings. The high AUC and associated diagnostic metrics affirm its capacity to serve as an early predictive tool for stratifying pediatric patients by risk level at the time of hospital admission or diagnosis.

Clinical Case application**.** To illustrate the practical application of the model, a clinical case is presented. A 13-year-old patient was admitted to the Municipal Children's Clinical Hospital in Ternopil with a diagnosis of COVID-19, bilateral lower lobe viral-bacterial pneumonia of moderate severity, and grade II respiratory failure.

Laboratory evaluation revealed the following biomarker levels:

- TNF-α: 95 pg/mL
- IL-6: 105 pg/mL
- Vitamin B_9: 2 ng/mL
- Vitamin A: 180 ng/mL
- Vitamin D: 15 ng/mL

Substitution of these values into the logistic regression equation yielded:

$$z = -3.0153 + 0.1413\times95 + 0.0047\times105 - 0.0543\times2 - 0.0002\times180 - 0.2773\times15$$
$$z = -3.0153 + 13.4235 + 0.4935 - 0.1086 - 0.036 - 4.1595 = 6.5976$$

The predicted probability was subsequently calculated as:

$$P = 1 / (1 + e^{-z}) = 1 / (1 + e^{-6.5976}) \approx 0.9986 \text{ (or } 99.86\%)$$

The model thus predicted a 99.86% probability of severe COVID-19 in this patient. The concurrent presence of deficient vitamin B_9, vitamin A, and vitamin D concentrations alongside markedly elevated TNF-α and IL-6 contributed substantially to the high-risk classification.

This case illustrates the model's value in real-time clinical decision-making. Measurement of serum vitamin B_9, vitamin A, and vitamin D concentrations alongside TNF-α and IL-6 provides a quantitative, evidence-based approach to identifying children at high risk of a severe COVID-19 course.

The logistic regression equation derived from this study identifies the most informative predictive variables and enables early, individualised risk estimation, thereby supporting triage decisions and proactive therapeutic interventions.

These results highlight the clinical utility of the proposed logistic regression model as a reliable and interpretable tool for identifying paediatric patients at risk of severe COVID-19, enabling timely intervention and resource prioritisation.

Discussion

The present study aimed to identify clinical and biochemical predictors of severe COVID-19 in children through a logistic regression model incorporating key proinflammatory cytokines and micronutrient concentrations. These findings confirm that elevated IL-6 and TNF-α concentrations are significantly associated

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with severe outcomes, consistent with previous reports implicating cytokine dysregulation in COVID-19 pathogenesis [3-5]. IL-6 is recognised as a central mediator of cytokine storm and is frequently elevated in patients with severe disease [5, 6]. The significance of TNF-α in pediatric COVID-19 is equally noteworthy; although most data derive from adult cohorts, several pediatric studies have highlighted its involvement in multisystem inflammatory syndromes [4, 5].These findings reinforce the value of both cytokines as early biomarkers of clinical deterioration.

Micronutrient deficiencies – particularly those of vitamin D, vitamin A, and folate (vitamin B_9) – also demonstrated strong associations with disease severity. These findings align with prior studies linking vitamin D deficiency to increased susceptibility to respiratory infections and adverse COVID-19 outcomes [7-12]. The immunomodulatory properties of vitamin D, including attenuation of pro-inflammatory cytokine release and enhancement of innate defences through antimicrobial peptides, likely underlie this protective effect [10, 11]. Vitamin A, recognised for its role in maintaining epithelial integrity and regulating immune responses, similarly emerged as a protective factor [15, 16]. Folate deficiency, previously associated with impaired immune function, was identified as an independent risk factor [18, 19]. Vitamins B_6 and B_{12} were not retained in the final model on grounds of statistical non-significance, although their broader immunological relevance is acknowledged.

Odds ratios derived from the model provided interpretable risk estimates: elevated IL-6, for example, was associated with an OR of 2.13, confirming its role as a strong positive predictor. The model achieved high diagnostic performance, with sensitivity of 87.5%, specificity of 93.3%, overall accuracy of 91.3%, and an AUC of 0.96-values considered excellent in predictive modeling [20]. Such performance is particularly important in pediatrics, where unnecessary interventions carry potential for harm, yet early identification of high-risk cases remains critical. Comparable performance has been reported for models integrating cytokine and nutritional parameters [6, 11, 13, 21].

The clinical utility of this approach was demonstrated through a real-life clinical case: in a 13-year-old patient presenting with elevated IL-6 and TNF-α concentrations alongside deficiencies of vitamins A, D, and B_9, the model predicted a 99.86% probability of severe COVID-19. This degree of individualised risk prediction illustrates the potential of biochemical profiling to guide decisions regarding monitoring intensity, hospitalisation, and targeted nutritional support.

Collectively, these findings emphasise the importance of evaluating immune-nutritional status in pediatric COVID-19 and support the rationale for early nutritional interventions as part of an integrated treatment strategy [14, 16, 18, 23]. Current evidence indicates that severe disease in children is also associated with immune dysregulation and the development of hyperinflammatory conditions, including multisystem inflammatory syndrome, further underscoring the role of immunometabolic disturbances in COVID-19 pathogenesis [25-28].

The proposed logistic regression model is statistically robust and clinically relevant, providing a practical tool for early risk stratification of severe COVID-19 in children. Beyond its applicability to pandemic preparedness and resource allocation [1, 2, 24], this integrative approach may serve as a basis for immunonutritional risk assessment in other infectious diseases affecting pediatric populations.

Conclusions

A multivariable logistic regression model was developed and validated to predict the risk of severe COVID-19 in children, incorporating pro-inflammatory cytokines (IL-6 and TNF-α) and micronutrient concentrations (vitamins A, D, and B_9). The model demonstrated excellent predictive performance: AUC = 0.96; sensitivity = 87.5%; specificity = 93.3%; overall accuracy = 91.3%.

Elevated IL-6 and TNF-α concentrations emerged as strong positive predictors of disease severity, consistent with their established role in cytokine storm syndromes. Conversely, deficiencies in vitamins D, A, and folate significantly increased the likelihood of severe disease, underscoring the importance of adequate micronutrient status for immune resilience. Vitamins B_6 and B_{12} were not retained in the final model on grounds of statistical non-significance, although their immunomodulatory roles are acknowledged.

Clinical relevance was further confirmed through a case-based application, demonstrating the model's capacity to estimate individual risk and inform targeted preventive and therapeutic decisions. The integration of cytokine and nutritional biomarkers provides a practical, interpretable approach to early risk stratification, enabling timely intervention and contributing to optimised pediatric COVID-19 management.

Future research perspectives. Further studies should address expansion of the patient cohort, external validation of the model across independent clinical centers, and evaluation of the effect of micronutrient status correction on disease course and model predictive performance.

Author contributions. H. A. Pavlyshyn – study concept and design, supervision, manuscript editing. O. V. Labivka – data collection and analysis, statistical analysis, manuscript preparation.

Conflict of interest. The authors declare no conflicts of interest.

Use of artificial intelligence. Artificial intelligence tools were employed during manuscript preparation for language editing and stylistic improvement and did not influence the interpretation of the study results.

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НЕОНАТОЛОГІЯ, ХІРУРГІЯ ТА ПЕРИНАТАЛЬНА МЕДИЦИНА
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ВЗАЄМОДІЯ ЦИТОКІНІВ І ВІТАМІНІВ ЯК ПРЕДИКТОРІВ ТЯЖКОСТІ COVID-19 У ДІТЕЙ: РОЗРОБКА ТА ВАЛІДАЦІЯ ЛОГІСТИЧНОЇ МОДЕЛІ

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Резюме.

Хоча більшість випадків COVID-19 у дітей мають легкий перебіг, у частини з них розвивається тяжке захворювання, зумовлене дисрегуляцією імунної системи та дефіцитом поживних речовин. Прозапальні цитокіни, такі як TNF-α та IL-6, відіграють центральну роль у гіперзапальних реакціях, тоді як дефіцит ключових вітамінів (A, D, B_6, B_9, B_{12}) може порушувати противірусний захист і регуляцію імунної відповіді. Однак комбінована прогностична цінність профілів цитокінів і мікронутрієнтів для прогнозування тяжкості захворювання у дітей залишається недостатньо визначеною.

Мета: побудувати та валідизувати багатофакторну модель логістичної регресії для прогнозування тяжкого перебігу COVID-19 у дітей на основі запальних та мікронутрієнтних біомаркерів.

Матеріали і методи. Клінічні та лабораторні характеристики були проаналізовані у 175 дітей із підтвердженим COVID-19. Для побудови прогностичної моделі було включено підгрупу з 112 дітей із повними даними щодо цитокінів і мікронутрієнтів. Аналізували рівні TNF-α, IL-6 та вітамінів B_9, A і D у сироватці крові. Покрокова логістична регресія дозволила визначити незалежні предиктори. Ефективність моделі оцінювали у навчальній (n=89) та тестовій (n=23) вибірках за допомогою аналізу ROC-кривої та стандартних класифікаційних показників. Комісія з біоетики Тернопільського національного медичного університету імені І. Я. Горбачевського МОЗ України (протокол № 79 від 07.11.2025) схвалила дослідження. Індивідуальна інформована згода на цей аналіз була отримана від усіх опікунів дітей. Статистичну обробку виконували з використанням програм Statistica 10.0, SPSS 26.0. Під час статистичної обробки отриманих даних для середніх значень розраховано їх 95% довірчий інтервал (95% ДІ), а критерієм достовірності для перевірки рівності медіан кількох вибірок є критерій Крускала-Уолліса (Н-критерій). Рівень статистичної значущості прийнято (P<0,05). Для побудови прогностичної моделі застосовували багатофакторну логістичну регресію з покроковим відбором змінних. Діагностичну ефективність оцінювали за допомогою ROC-аналізу (AUC, чутливість, специфічність). Для порівняння груп використовували критерій Крускала–Уолліса. Рівень статистичної значущості приймали при $p < 0,05$. Дане дослідження є фрагментом науково – дослідної роботи «Оптимізація діагностики клініко-патогенетичних характеристик коронавірусної інфекції COVID-19 у дітей з коморбідною патологією та особливості лікування « (державна реєстрація № 0123U100064, термін виконання 2023-2025 рр.).

Результати. Підвищені рівні IL-6 та TNF-α разом із дефіцитом вітамінів B_9, A та D були значущими предикторами тяжкості захворювання (p<0,05). Модель продемонструвала відмінну дискримінаційну здатність: площа під ROC-кривою (AUC) становила 0,96, чутливість – 87,5%, специфічність – 93,3%, загальна точність – 91,3%, що забезпечує надійне розмежування між легкими та тяжкими випадками.

Висновок. Ця модель логістичної регресії є надійним та інтерпретованим інструментом для раннього виявлення дітей із ризиком розвитку тяжкого перебігу COVID-19. Інтеграція цитокінових та мікронутрієнтних біомаркерів сприяє персоналізованим втручанням і може покращити клінічне прийняття рішень у педіатричній практиці.

Ключові слова: COVID-19; діти; тяжкість захворювання; логістична регресія; цитокіни; вітаміни.

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