ORIGINAL ARTICLE

Neuron-specific enolase level research in children with COVID-19

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ABSTRACT

Introduction

COVID-19 is a relevant issue for scientists around the world. Considering the development of numerous complications due to COVID-19, the search for diagnostic approaches for the timely detection of probable lesions of the nervous system is urgent.

Objectives

was to determine the diagnostic and prognostic value of the NSE marker in children with COVID-19 regarding the complicated course and symptoms of nervous system damage.

Materials and methods

We conducted a cohort, observational, retrospective study involving 88 children aged 1 month to 18 years with laboratory-confirmed COVID-19 by PCR. Blood serum was collected for the study to assess the level of the neurobiomarker NSE by enzyme immunoassay. We divided patients into two cohorts according to the course of the disease - the main one, which included 42 patients with a complicated course of COVID-19, and a control group - 46 patients with an uncomplicated course of the disease. The study protocol was approved by the Local Ethics Committee of the hospital. ***Results:*** In the patients of the control group, NSE was observed at the level of 12.1±1.2 μg/l, while in the children of the main one, the indicator reached 16.9±1.5 μg/l (p=0.087). Increase in the level of NSE above 15 μg/L associated with a significant increase in the risk of the appearance of clinical symptoms of damage to the nervous system and a complicated course in children with COVID-19 (p<0.05). We noted a tendency towards an increase in NSE with increased ESR indicators >10mm/h (p<0.1), a decrease in PTI <85% (p=0,03) and an increase in D-dimer >2.5mg/l (p<0.1).

Conclusions

We found the diagnostic and prognostic value of the NSE marker in children with COVID-19 regarding the complicated course and symptoms of nervous system damage.

Keywords

COVID-19; SARS-CoV-2;neuromarker, NSE; children, nervous system; laboratory-instrumental diagnostics.

INTRODUCTION:

The coronavirus infection (COVID-19) remains an urgent problem for scientists around the world. There are 767 million laboratory-confirmed cases of COVID-19 worldwide as of early June 2023. Almost 7 million of them were fatal. During this period, there were registered 5.5 million laboratory-confirmed cases and 112,000 deaths in Ukraine [1].

The clinical picture of COVID-19 covers a wide spectrum of organ and system damage. The most

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common symptoms in adults include fever (74.8%), non-productive cough (42.2%), fatigue (13.1%), sore throat (12.8%) and shortness of breath (11.7%). Less common symptoms are nasal congestion, chest discomfort, muscle pain, chills, headache, diarrhea, expectoration, and joint pain. As for children, their most prominent symptoms are fever (100%), cough (90%), diarrhea (80%), vomiting (75%), sore throat (65%), shortness of breath (60%), headache (55%).), abdominal pain (50%), malaise and weakness (50%) [2,3].

The constant mutation of the virus causes the wave-like course of the disease. The SARS-CoV-2 Omicron variant of the coronavirus was first identified in November 2021, had a large number of mutations and superseded most previous circulating strains. The most common symptoms of this variant of COVID-19 among adults were fatigue (81%), headache (76%), sneezing (74%) and nasal congestion (73%). Fever (72%), fatigue (65%), headache (63%) and sore throat (56%) were observed among infected children. Children had significantly fewer symptoms than adults (median 8 vs. 11) ($p<0.001$). However, they were significantly more likely to report fever (72% vs. 68%, $p=0.006$) and decreased appetite (49% vs. 42%, $p<0.001$) [4].

The spectrum of complications of COVID-19 covers a wide range of extrapulmonary manifestations, including damage to the nervous system, kidneys, liver, skin, as well as disorders of the gastrointestinal tract, cardiovascular, endocrine systems [5,6].

According to data from numerous published literature sources, the frequency of neurological symptoms in children and adults with COVID-19 varies widely [5,7-9]. There are nonspecific mild symptoms, that manifested with headache (8–42%), myalgia and/or fatigue (11–44%), dizziness (12%), anorexia (40%), anosmia and ageusia (5%), and more severe manifestations of COVID-19, such as confusion or impaired consciousness (8–9%), acute stroke (6%), and cases of acute inflammatory demyelinating polyneuropathy (Guillen-Barre syndrome), meningoencephalitis, posterior reversible encephalopathy syndrome, and acute necrotic encephalopathy [9-17].

According to a recent multicenter study by Panda et al (2021), 7% of children with COVID-19 had neurological complications. More than 15,000 patients with COVID-19 aged 2 months to 17 years who were hospitalized in 52 US children's hospitals between March 2020 and March 2022 were involved in the study. Almost 4% of patients had neurological complications in the form of febrile convulsions and 2.3% had convulsions that were not associated with fever. Encephalopathy was also observed in 2.2% of patients. The authors noted that neurologic manifestations were associated with increased frequency of intensive care unit admissions and longer stays, as well as overall longer hospital stays and higher financial costs [18].

The mechanism of central nervous system (CNS) damage in COVID-19 is demonstrated in the work of Stafstrom & Jantzie (2020), where the researchers detailed four potential mechanisms of nervous system involvement: direct neurotropic or neuroinvasive effect of SARS-CoV-2; secondary damage due to systemic inflammatory reactions caused by a viral infection; secondary damage associated with the vascular and prothrombotic effect of a viral infection on the vascular system of the CNS or peripheral nervous system (PNS); immune-mediated parainfectious or postinfectious autoimmune action in response to a viral infection. Scientists describe the possible nervous system damage as follows: SARS-CoV-2 attaches to the olfactory epithelium using the angiotensin-converting enzyme (ACE-2) receptor. When the virus enters a cell, it replicates and induces a massive immune response that leads to an excessive release of cytokines. SARS-CoV reaches the CNS retrogradely through the blood-brain barrier (BBB) or pathways of V, I, VII, IX, or X pairs of cranial nerves. A variety of neurological signs and symptoms of CNS or PNS lesions occur after reaching CNS nuclei, including the brainstem and cortex [19].

Considering the long course of the pandemic and the development of numerous complications due to COVID-19, it is necessary to search for diagnostic approaches in order to timely identify possible lesions of the nervous system Biomarkers are used by the scientific community for this purpose. These are specific molecules found in the blood and cerebrospinal fluid that can indicate damage to the nervous system and BBB. One of these compounds is neuron-specific enolase (NSE) which is considered a potential biomarker of CNS damage.

NSE is an enzyme involved in glycolysis processes. This enzyme is found in the cytoplasm and dendrites of neurons and neuroendocrine cells. It is important in the diagnosis of lesions of the nervous system of various origins, because entering the blood through damaged plasma membranes of brain cells, it indicates significant

structural-functional and destructive cytomembrane disorders [20].

To date, NSE has been used for oncology of neuroendocrine origin detection, especially such as small cell lung cancer and neuroblastomas [20]. In addition, this marker has proven prognostic value for patients with severe brain injury, septic shock, and sepsis- related encephalopathy [22,23].

The important value of the marker was also determined for the diagnosis of brain damage in children. In 2016, an observational study was conducted in Iran among 62 children who were admitted to the emergency department of a hospital with blunt traumatic brain injury due to traffic accidents, household or sports injuries. The researchers collected serum from the patients within the first 6 hours after the incident to assess NSE levels. In the course of the work, the scientists established a higher level of NSE in the blood serum of patients with brain injuries and suggested using this marker as a highly accurate diagnostic tool for assessing the presence of brain lesions in infants associated with head injuries [24].

In a similar study, the prognostic value of the NSE marker in children with acute brain injury was determined. Results were correlated with the Glasgow Score and/or GOS-Extended Pediatric. Higher concentrations of NSE were observed in children younger than 4 years compared to older children. Using binary logistic regression, the influence of NSE on the prognostic value of the consequences of brain injury was confirmed, so the researchers concluded that the NSE marker has prognostic value in the diagnosis of brain injuries in children [25].

Another research was published by Ukrainian scientists in 2018. Pypa et al (2018) investigated the diagnostic value of NSE as an indicator of neuronal damage in children with acute meningitis. Researchers suggest using NSE as a biochemical marker of the severity of neuronal damage, as well as a prognostic marker of the development of CNS complications [26].

Due to the spread of COVID-19, neuromarkers are being actively studied in this aspect of the disease as predictors of severe course and nervous system damage. In one of the first pilot studies, scientists propose the NSE marker as a predictor of clinical severity of COVID-19. Sahin BE et al (2022) conducted a prospective study involving 20 healthy individuals and 59 patients with laboratory-confirmed COVID-19 in whom serum NSE levels were determined by enzyme-linked immunosorbent assay (ELISA). As a result of their work, the authors found a higher level of NSE in blood serum in the group of patients with a severe course than in the group without complications of COVID-19 ($p=0.034$). Elevated serum NSE correlated with disease severity independent of accompanying neurological symptoms in patients with COVID-19 [27].

In another work, American scientists determined the level of brain damage markers (among which was NSE), markers of endothelial damage and inflammation in plasma samples of 57 patients of the main group, which included persons hospitalized with a diagnosis of COVID-19 and 20 healthy controls. As a result, the level of NSE and other markers of brain damage, endothelium and inflammation were significantly increased ($p<0.05$) in the COVID-19 cohort compared to the control group. As a result of the bioinformatic analysis, a strong positive relationship between the markers in the COVID-19 cohort compared to the control group was found, so the authors noted that this reliable relationship is a confirmation of brain and endothelium damage [28].

The use of NSE and other biomarkers for diagnosing and predicting the severity of COVID-19 in children is a fairly new and little-studied field that requires additional research. Therefore, we wanted to determine the level of NSE in children and evaluate the dependence of the enzyme level on the severity of COVID-19 and symptoms of the nervous system damage.

The purpose of the study was to determine the diagnostic and prognostic value of the NSE marker in children with COVID-19 regarding the complicated course and symptoms of the nervous system damage.

MATERIALS AND METHODS

Study design and data gathering

We retrospectively investigated 945 cases of laboratory-confirmed COVID-19 by the PCR method in children aged 1 month to 18 years who underwent inpatient treatment in the Kyiv City Children's Clinical Infectious Diseases Hospital in Kyiv, Ukraine during 2020-2022. Among them, to conduct a cohort, observational, retrospective study, the level of NSE enzyme was determined in 88 patients in a complex of routine general clinical examinations. According to

age groups, we divided children into 4 main categories - from birth to 12 months, from 1 to 6 years, from 6 to 10 years and from 10 to 18 years. We also divided patients into two cohorts according to the course of the disease - the main one, which included 42 patients with a complicated course of COVID-19, and the control group - 46 patients with an uncomplicated course of the disease. In the study, we took into account the main laboratory parameters (general, biochemical blood analysis, coagulogram), patient complaints and clinical picture.

During the comprehensive examination of the patients during the first day of their stay in the hospital, blood serum of the patients was collected for its further examination of the neurobiomarker NSE level by enzyme immunoassay. Fujirebio's «CanAg NSE EIA kit» with a working measurement range of 1-150 µg/L for the NSE marker was used.

Eligibility criteria

The inclusion criteria for this study were: children under the age of 18 who were undergoing inpatient treatment and had laboratory-confirmed COVID-19. The study did not include patients older than 18 years, other infectious pathology, an unconfirmed or refuted diagnosis of COVID-19, the presence of congenital or concomitant lesions of the nervous system, as well as a history of cancer in patients.

Statistical analysis

In the study, we used statistical, analytical and empirical research methods. Personal data of patients were not used for statistical processing. To calculate the obtained results, we used the statistical program «Statistical software EZR v.1.54». The median (M) and standard deviation (SD) were determined. The reliability of the difference between non-parametric indicators was determined using the Chi-square test. The difference is accepted as significant when the error value is $p<0.05$. We also conducted an interval assessment of the distribution, multiple comparisons, calculated the Pearson correlation coefficient and applied the Wilcoxon W-test and determined the prognostic value of the marker by calculating the odds ratio.

ETHICAL CLEARENCE:

The work is a fragment of the research project «Modern features of acute neuroinfections in children»of the Department of Children's Infectious Diseases of Bogomolets National Medical University, state registration number 0119U103914, date of implementation 2020-2023.

Data gathering was carried out in the clinic of the Department of Pediatric Infectious Diseases of Bogomolets National Medical University and idea owners of this study are employees of the Department of Children's Infectious Diseases.

The study protocol of the research was approved by the Biomedical Ethics Committee of Kyiv City Children's Clinical Infectious Diseases Hospital (Ethical approval number № 25 dated 01.12.2022). Since NSE was included in the list of routine laboratory tests, informed consent of parents and children was not performed.

RESULTS

Table 1 shows the age and gender indicators of the study groups. According to the results of the study, no significant difference was observed in terms of gender characteristics between the patients of the complicated and uncomplicated course groups (p=0.8).

Table 1. Age-gender characteristics of the study groups, abs. (%)

Criteria	Control group (n=46)	Main group (n=42)	P
Age			
0 - 12 months	23 (50)	7 (16,7)	p<0,001
1 - 6 years	16 (34,8)	13 (30,9)	p=0,7
6 - 10 years	3 (6,5)	6 (14,3)	p=0,23
10 - 18 years	4 (8,7)	16 (38,1)	p=0,002
Gender			
Male	25 (54,3)	22 (52,4)	p=0,8
Female	21 (45,7)	20 (47,6)	p=0,8

According to the age structure (Table 1), the control group was dominated by patients under 12 months, there were 23 (50%) cases, $p<0.001$. In the main cohort of patients with a complicated course, children aged 10-18 years prevailed, there were 16 (38.1%) cases. The age group of 6-10 years was the least numerous, accounting for 3 (6.5%) patients in the control group and 6 (14.3%) in the main one. When comparing the control and primary groups, the most significant was the age of children from birth to 12 months (50% in the

primary versus 17.7% in the control groups), p<0.001 and adolescents (8.7% in the primary group versus 38.1% in the control group), p=0.002. All children were discharged with improved condition, no deaths were registered among the study groups.

During the COVID-19 pandemic from 2020 to 2022, we studied 945 laboratory-confirmed cases of the disease in children who underwent inpatient treatment in our hospital. Among them, a complicated course was observed in 232 (24.5%) patients. The detailed structure of the complicated process is shown in Figure 1.

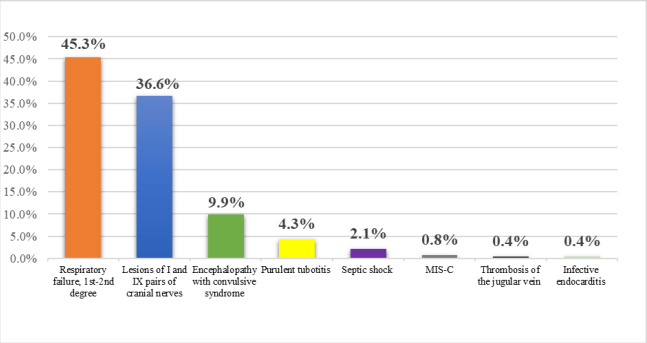

Figure 1. Structure of the complicated course in patients with COVID-19 (2020-2022 period)

In the structure of the complicated course in children with COVID-19 (Fig. 1), respiratory manifestations prevailed in the form of respiratory failure of the 1st-2nd degree, which was observed in 105 (45.3%) cases. The next most frequent complications were from the nervous system in the form of lesions of I and IX pairs of cranial nerves, 85 (36.6%) and encephalopathy with convulsive syndrome - 23 (9.9%) cases. Also, among the complications, purulent tubotitis was recorded in 10 (4.3%) patients, septic shock in 5 (2.1%), two cases of multisystem inflammatory syndrome in children (MIS-C) - (0.8%) and one case each complications from the cardiovascular system in the form of thrombosis of the right internal jugular vein and infective endocarditis of the aortic valve (0.4% for each).

Among the patients of the cohort that we studied regarding the level of the NSE marker, complications were observed in 42 (47.7%) children and, accordingly, the course without complications was observed in 46 (52.3%) patients (p<0.001). Complications were confirmed by the data of clinical, laboratory and instrumental examinations, characterized primarily by respiratory manifestations in the form of respiratory failure of the 1st-2nd degree in 23 (54.8%) cases, acute stenotic laryngotracheitis - 6 (14.2%), purulent tubotitis in 1 (2.4%) patient, as well as damage from the nervous system: encephalopathy with convulsive syndrome in 4 (9.5%) children and damage to I and IX pairs of cranial nerves in 8 (19.1%) patients.

Neurological symptoms in children with COVID-19 were observed in 46 (52%) cases, that characterized by headache in 24 (52%) children, myalgia -8 (17%), ageusia/anosmia - 8 (17%) and encephalopathy with convulsive syndrome in 6 (13%) cases.

In order to perform a statistical calculation to compare the main and control groups, we carried out an analysis of the interval assessment of the NSE marker in patients with COVID-19 in both groups. The results are presented in Table 2.

The range of reference values for NSE is less than 10 µg/L, but the manufacturers of this scientific kit emphasize that all values are individual and should be estimated in conjunction with the results of other laboratory and instrumental indicators. According to the calculations, in patients of the control group, we observed NSE at the level 12.1±1.2 µg/l, while in children of the main group, the indicator reached 16.9±1.5 µg/l (p=0.087).

According to the interval assessment of the biomarker level, patients of the main group had higher NSE values than patients of the control one. When comparing the central tendencies for the two samples by the Wilcoxon W-test, a significant difference was found, p=0.02. The obtained results demonstrated that patients with a complicated course of COVID-19 had a significantly higher level of NSE, compared to children who did not have a complicated course of COVID-19.

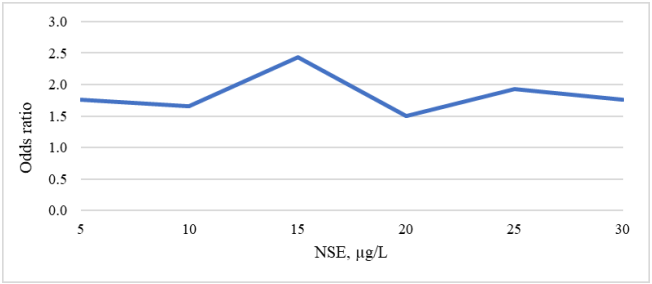

Figure 2. Prognostic value of NSE for the development of neurological symptoms in children with COVID-19

Table 2. Interval evaluation of the NSE marker in patients with COVID-19

Indicator	Group	Me±SD	Minimum	Maximum	Left (95% CI)	Right (95% CI)
NSE	Control group	12,1±1,2	4,8	28,5	9,1	14,7
	Main group	16,9±1,5	5,3	31,2	12,3	20,9

Among the children of the studied cohort, the level of NSE varied widely. However, even in patients with an elevated level of the investigated marker, clinical symptoms of nervous system damage were not observed in every case. We assumed the existence of a relationship between the level of NSE and the risk of the appearance of clinical symptoms of CNS damage. To test this hypothesis, we determined the odds ratio (OR) for ranges of NSE values in increments of 5 µg/l. The results are shown in Figure 2.

From the given data in Figure 2, an NSE value above 15 µg/L had a direct relationship with the odds ratio value. This gives reason to believe that an increase in the level of NSE associated with an increase in the risk of the appearance of clinical symptoms of the nervous system damage ($p<0.05$). To confirm the above results, we calculated the correlation between NSE and symptoms of nervous system damage in COVID-19 according to Pearson's test. Higher values of NSE were more often detected with existing neurological symptoms in children. The value of the correlation coefficient r=0.171 (95% CI -0.04-0.37) was statistically significantly different from 0 ($p<0.1$).

According to the obtained results of the odds ratio of the neurological symptoms development in children with COVID-19 and the level of NSE, as well as taking into account the involvement of the nervous system in the pathogenesis of COVID-19, we decided to investigate the relationship of the NSE marker with the severity of the course of the disease and the development of complications in COVID-19. To test this hypothesis, we determined the odds ratio for ranges of NSE values in increments of 5 µg/l for the development of such complications in children as respiratory failure of the 1st-2nd degree, acute stenosing laryngotracheitis, purulent tubotitis, encephalopathy with a convulsive syndrome, and lesions of the I and IX pairs of cranial nerves (Figure 3).

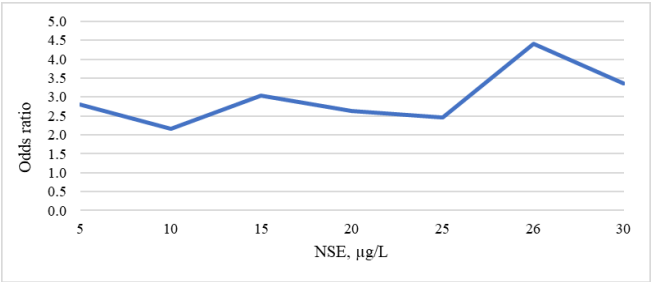

Figure 3. Prognostic value of NSE regarding the development of a complicated course of COVID-19 in children

The results of the study shown in Figure 3 demonstrate an association of NSE at a level above 15 µg/L with a clear trend toward an increase in the odds ratio for a complicated course of the disease. Therefore, an increase in the level of NSE associated with an increase in the risk of a complicated course of COVID-19 in children ($p<0.05$). According to the Pearson test, a correlation between NSE and the complicated course of COVID-19 was also confirmed ($p=0.02$). Higher NSE values were significantly more common in severe disease with complications in children. The value of the correlation coefficient r=0.258 (95% CI 0.05-0.44) was statistically significantly different from 0.

We also analyzed the relationship between the level of NSE and the main hematological changes associated with the severity of the disease, in particular with the severity of the inflammatory reaction (increased level of leukocytes (>9 G/L), increased C-reactive protein (CRP>6 mg/L) increased level of ESR (>10 mm/h)), liver damage marker ALT (>35 IU/l) and indicators of coagulopathy (increased level of D-dimer (>2.5mg/l), increased fibrinogen (>4g/l) and decreased level prothrombin index (PTI) (<85%)). The results are shown in Figure 4.

In Figure 4 we depicted a forest diagram with the calculation of the odds ratio and confidence intervals of the main hematological changes in children with an NSE level higher than 10 µg/l. As a basis, we chose

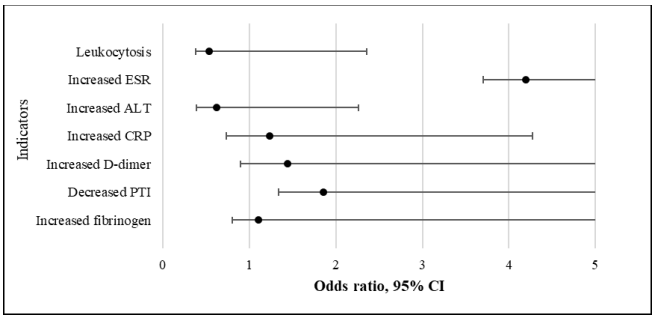

Figure 4. Adjusted odds ratios for hematological changes with an elevated level of NSE among hospitalized patients with COVID-19

exactly this starting level of the marker, since the level up to 10 µg/l is indicated by the manufacturers of the set as a range of reference values. We did not find a clear linear relationship between NSE and laboratory parameters, which is most likely due to the small sample size of patients. But there is a tendency to increase NSE with increased ESR >10 mm/h ($p<0.1$), reduction of PTI <85% ($p=0.03$) and increase of D-dimer >2.5 mg/l ($p<0.1$). Therefore, patients with these laboratory findings statistically had the greatest chance of obtaining a higher level of NSE.

We also checked the correlation of the NSE biomarker level with D-dimer, PTI and ESR according to the Pearson test. When conducting a study of the correlation of a neurobiomarker with PTI, a negative linear correlation was determined ($p=0.03$). An increase in the NSE indicator was significantly more often observed with a decrease in PTI. The value of the correlation coefficient for NSE r =-0.12 (95% CI -1-0.07) was statistically significantly different from 0.

The correlation between the neurobiomarker NSE and D-dimer was also confirmed ($p<0.1$). The value of the correlation coefficient for NSE r=0.153 (95% CI -0.0594-0.352) was statistically significantly different from 0. PTI and D-dimer in COVID-19 are predictors of the severity of the condition, therefore the correlation of the neuromarker with these indicators may indicate endothelial damage and to confirm the role of the vascular system in the pathogenesis of nervous system damage in COVID-19.

A trend toward correlation was also established between ESR and NSE ($p<0.1$). Elevation of the investigated marker was significantly more common in patients with a higher level of ESR. The value of the correlation coefficient r=0.152 (95% CI -0.06-0.35) was statistically significantly different from 0. Since the increase in ESR is an indicator of the inflammatory process, the connection with NSE in this case may indicate the influence of the inflammatory component on increased secretion of NSE enzyme in the acute phase of the disease.

DISCUSSION

We have dedicated this research to studying the level of the NSE marker and evaluating the presence of a relationship with the severity of the course and symptoms of the nervous system of COVID-19. Determining the role of biomarkers of COVID-19 in children has an important prognostic and diagnostic value and is an actual area of research. The diagnostic value involves the quick and accurate establishment of the presence of the nervous system damage and the determination of the severity degree, which allows for timely treatment and prevention of a complicated course of the disease. The prognostic value consists in determining the risk of developing severe forms of damage to the central nervous system and predicting the consequences.

The significance of NSE is widely described in the literature related to oncodiagnostics [29,30]. However, scientists are also studying the use of NSE in lung diseases. Published data describe the value of NSE in the treatment and diagnosis of infectious lung diseases, acute and chronic lesions, and pulmonary nodules [31,32].

We sought to investigate the value of NSE in pediatric coronavirus infection, given its reported value in the aspect of lung damage. We noted that there are no organ-specific diagnostic markers for COVID-19 in children, and taking into account its multisystem damage, we assumed that an increase in the level of the NSE enzyme would be associated with a severe course of the disease and symptoms of damage to the nervous system. Researchers around the world are already actively studying the feasibility of using NSE for COVID-19. In particular, Battaglini D. et al (2022) in their review of biomarkers in the diagnosis and prediction of the consequences of COVID-19, suggest the use of NSE in the diagnostic algorithm for the assessment of pulmonary function and neurological disorders in patients with COVID-19 [33].

According to the results of our work, higher rates of NSE were observed in the group with a complicated course, compared to the mild form of COVID-19 in

children. Complications were observed from the side of the respiratory system in the form of respiratory failure of the 1st-2nd degree and stenosing laryngotracheitis. Similar to our study, in the work of Italian scientists, NSE was determined at different severity of COVID-19. Significantly higher NSE values (P<0.05) were found in patients with severe form of COVID-19, accompanied by respiratory failure and shortness of breath. The authors evaluate the obtained research results as a basis for further study of the prospective significance of NSE as a key marker indicating the spread of COVID-19 [34].

Another publication assessed brain damage by associating with increased serum levels of the neurobiomarkers NSE and S100 in patients with COVID-19 in Brazil. Patients were divided into groups - healthy individuals, patients with a mild course of COVID-19, as well as a severe form of the disease that required additional oxygenation or hospitalization in the intensive care unit. The results showed a significant positive association between patients with severe disease and serum neuromarker expression (p=0.0403). The level of NSE in blood serum was significantly increased in this group of patients compared to a group of healthy individuals (p<0.0001) and patients with mild symptoms of COVID-19 (p<0.0001) [35]. The results of this work are consistent with our study, as we also found a relationship of the marker with the severity of the condition and the symptoms of the nervous system damage, although our patient cohort lacked healthy individuals and critically ill patients.

Despite the fact that our work had a number of limitations, including the retrospective nature of the work, the availability of data from only one center, the lack of information on patients who were in outpatient treatment and in the post-covid period, we plan to continue the analysis of the NSE marker in the context of COVID-19 We are going to increase the sample to trace the regularity of the results already obtained. We are convinced that in the future it will provide an opportunity to find out the trends and prospects of damage to the nervous system in children and to optimize diagnostic algorithms.

CONCLUSIONS:

In conclusion, based on our research, the diagnostic and prognostic value of the NSE marker in children with COVID-19 regarding the complicated course and symptoms of nervous system damage was revealed. We established a reliable association between increased NSE with neurological symptoms and severity of COVID-19. An increase in the level of NSE above 15 μg/l correlates with an increased risk of clinical symptoms from the nervous system and a complicated course in children with COVID-19 (p<0.05). To confirm the role of NSE in the severity of the course of the disease, we analyzed the relationship of the marker with hematological changes that demonstrate the presence of a pronounced inflammatory process, coagulopathy or liver damage. And although a clear linear relationship of the marker with laboratory indicators was not found, we noted a tendency to increase NSE with increased ESR >10mm/h (p<0.1), decreased PTI <85% (p=0.03) and increased D-dimer >2.5mg/l (p<0.1).

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Declaration of interest statement

The authors declare that they have no conflict of interest.

Author Contributions statement

All of the authors contributed equally in manuscript work & production.

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References

1. World Health Organization. WHO Coronavirus (COVID-19) Dashboard. 2023 https://covid19.who.int/
2. Melo MM, Neta MMR, Neto ARS, Carvalho ARB, Magalhães RLB, et al. Symptoms of COVID-19 in children. *Braz J Med Biol Res* 2022;**55**:e12038. doi: 10.1590/1414-431X2022e12038.
3. Ibrahim MS, Hassan NM, Aziz AA, Yusoff HM, Esa NK, et al. Clinical manifestations of COVID-19; what have we learned from the global database? *Bangladesh Journal of Medical Science*,2022; **21**(3): 702–709. https://doi.org/10.3329/bjms.v21i3.59588.
4. Petersen MS, Í Kongsstovu S, Eliasen EH, Larsen S, et al. Clinical characteristics of the Omicron variant - results from a Nationwide Symptoms Survey in the Faroe Islands. *Int J Infect Dis* 2022;**122**:636-643. doi: 10.1016/j.ijid.2022.07.005..
5. Gupta, A., Madhavan, M.V., Sehgal, K. et al. Extrapulmonary manifestations of COVID-19. Nat Med 2020; **26**:1017–1032. https://doi.org/10.1038/s41591-020-0968-3
6. Akter F, Ahmad R, Haque M. Endocrine Flawed In COVID-19 Era. *Bangladesh Journal of Medical Science*, 2021; **20**(5): 49–64. https://doi.org/10.3329/bjms.v20i5.55416
7. Kempuraj D, Selvakumar GP, Ahmed ME, Raikwar SP, Thangavel R, et al. COVID-19, Mast Cells, Cytokine Storm, Psychological Stress, and Neuroinflammation. *Neuroscience* 2020; 1–13. doi: 10.1177/1073858420941476.
8. Misra S, Kolappa K, Prasad M, et al. Frequency of Neurologic Manifestations in COVID-19: A Systematic Review and Meta-analysis. *Neurology* 2021; **7**: e2269-e2281. doi: 10.1212/WNL.0000000000012930.
9. O'Loughlin L, Toledo NA, Budrie L, Waechter R, Rayner J. A systematic review of severe neurological manifestations in pediatric patients with coexisting SARS-CoV-2 infection. *Neurol Int* 2021; **13**:410–27.
10. Franceschi AM, Ahmed O, Giliberto L, Castillo M. Hemorrhagic posterior reversible encephalopathy syndrome as a manifestation of COVID-19 infection. *AJNR Am. J.* Neuroradiol 2020. https://doi.org/10.3174/ajnr.A6595.
11. Antoon JW, Hall M, Leigh M. Howard, et al. COVID-19 and Acute Neurologic Complications in Children. *Pediatrics* 2022; **150** (5): e2022058167. 10.1542/peds.2022-058167
12. Mao L, Jin H, Wang M, Hu Y, Chen S et al. Neurologic Manifestations of Hospitalized Patients With Coronavirus Disease 2019 in Wuhan, China. *JAMA Neurol* 2020; **77**(6):683-690. doi: 10.1001/jamaneurol.2020.1127.
13. Moriguchi T, Harii N, Goto J, Harada D, Sugawara H, et al. A first case of meningitis/encephalitis associated with SARS-Coronavirus-2. *Int J Infect Dis* 2020; **94**:55-58. doi: 10.1016/j.ijid.2020.03.062.
14. Poyiadji N, Shahin G, Noujaim D, Stone M, Patel S, Griffith B. COVID-19-associated Acute Hemorrhagic Necrotizing Encephalopathy: Imaging Features. *Radiology* 2020; **296**(2):E119-E120. doi: 10.1148/radiol.2020201187.
15. Yaghi S, Ishida K, Torres J, Mac Grory B, Raz E et al. SARS-CoV-2 and Stroke in a New York Healthcare System. *Stroke* 2020; **51**(7):2002-2011. doi: 10.1161/STROKEAHA.120.030335.
16. Zhao, H., Shen, D., Zhou, H., Liu, J. & Chen, S. Guillain-Barré syndrome associated with SARS-CoV-2 infection: causality or coincidence? *Lancet Neurol* 2020; **19**: 383–384.
17. Parry ZA, Bumb SS, Kumar S, Bhatt R, Irfan M, Bhatt P. Neurological Manifestations of Covid-19 Hospitalized Patients In the state of Punjab, India. *Bangladesh Journal of Medical Science*,2021; **20**(5): 155–161. https://doi.org/10.3329/bjms.v20i5.55414.
18. Panda PK, Sharawat IK, Panda P, Natarajan V, Bhakat R, Dawman L. Neurological Complications of SARS-CoV-2 Infection in Children: A Systematic Review and Meta-Analysis. *J Trop Pediatr* 2021; **67**(3):fmaa070. doi: 10.1093/tropej/fmaa070.
19. Stafstrom CE, Jantzie LL. COVID-19: Neurological Considerations in Neonates and Children. *Children (Basel)*. 2020 Sep 10;**7**(9):133. doi: 10.3390/children7090133.
20. Huang C, Wang Y, Li X, Ren L, Zhao J, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* 2020; **395**(10223):497-506. doi: 10.1016/S0140-6736(20)30183-5.
21. Budnyuk OO., Kartashov OA., Artemenko VYU. Neyron-spetsyfichna enolaza yak marker poshkodzhennya holovnoho mozku ta efektyvnosti neyroprotektornoyi terapiyi pry hostriy tserebral′niy nedostatnosti. Nevrolohiya. Neyrokhirurhiya. *Psykhiatriya* 2016; **579**:8-10.
22. Nguyen DN, Spapen H, Su F, et al. Elevated serum levels of S-100beta protein and neuron-specific enolase are associated with brain injury in patients with severe sepsis and septic shock. *Crit Care Med* 2006; **34**:1967–74.
23. Olivecrona Z, Bobinski L, Koskinen LO. Association of ICP, CPP, CT findings and S-100B and NSE in severe traumatic head injury. Prognostic value of the biomarkers. *Brain Inj* 2015; **29**:446–54
24. Mozafari J, Motamed H, Hanafi MG, Fatehifar B. The Diagnostic Value of Neuron-Specific Enolase in Children with Mild Blunt Trauma Requiring Cranial CT Scan. *Open Access Emerg Med* 2020; **12**:1-5. https://doi.org/10.2147/OAEM.S223179
25. Bavishi C., Bonow R.O., Trivedi V., et al. Acute myocardial injury in patients hospitalized with COVID-19 infection: a

review. *Prog Cardiovasc Dis* 2020; **63**(5):682–689.

26. Pypa L.V., Svistilnik R.V., Moskovko G.S., Lysytsia Yu.M., Murhina M.M. Neuron specific enolase as a possible indicator of neuron damage in children with acute meningitis. *Zaporozhye Medical Journal* 2018; **20**:3(108).C. 354-358.
27. Sahin BE, Celikbilek A, Kocak Y, Ilanbey B, Saltoglu GT, et al. Neurological symptoms and neuronal damage markers in acute COVID-19: Is there a correlation? *A pilot study*. *Journal of Medical Virology* 2022; **95**(1): https://doi.org/10.1002/jmv.28240
28. Savarraj J, Park ES, Colpo GD, Hinds SN, Morales D, et al. Brain injury, endothelial injury and inflammatory markers are elevated and express sex-specific alterations after COVID-19. *J Neuroinflammation* 2021; **18**(1):277. doi: 10.1186/s12974-021-02323-8.
29. Gambichler T, Abu Rached N, Susok L, Becker JC. Serum neuron-specific enolase independently predicts outcomes of patients with Merkel cell carcinoma. *Br J Dermatol* 2022; **187**(5):806-808. doi: 10.1111/bjd.21717.
30. Huang Z, Xu D, Zhang F, Ying Y, Song L. Pro-gastrin-releasing peptide and neuron-specific enolase: useful predictors of response to chemotherapy and survival in patients with small-cell lung cancer. *Clin Transl* Oncol 2016; **18**:1019–25. 10.1007/s12094-015-1479-4.
31. Barouchos N, Papazafiropoulou A, Iacovidou N, Vrachnis N, Barouchos N, et al. Comparison of tumor markers and inflammatory biomarkers in chronic obstructive pulmonary disease (COPD) exacerbations. *Scand J Clin Lab Invest* 2015; **75**(2):126–32. 10.3109/00365513.2014.992944
32. Xu CM, Luo YL, Li S, Li ZX, Jiang L, et al. Multifunctional neuron-specific enolase: its role in lung diseases. *Biosci Rep* 2019; **39**(11): BSR20192732. 10.1042/BSR20192732
33. Battaglini D, Lopes-Pacheco M, Castro-Faria-Neto HC, Pelosi P, Rocco PRM. Laboratory Biomarkers for Diagnosis and Prognosis in COVID-19. *Front Immunol* 2022; **13**:857573. doi: 10.3389/fimmu.2022.857573.
34. Cione E, Siniscalchi A, Gangemi P, Cosco L, Colosimo M, et al. Neuron-specific enolase serum levels in COVID-19 are related to the severity of lung injury. *PLoS One* 2021; **16**(5):e0251819. doi: 10.1371/journal.pone.0251819.
1. Silva RC, da Rosa MM, Leão HI. et al. Brain damage serum biomarkers induced by COVID-19 in patients from northeast Brazil. *J. Neurovirol* 2023. https://doi.org/10.1007/s13365-023-01119-1