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DETERMINATION OF NEUROBIOMARKERS IN CHILDREN WITH COVID-19

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The coronavirus infection (COVID-19) pandemic has become a real challenge for medical professionals and researchers, given the frequency of deaths and complications. Damage to the nervous system is one of the most common. The frequency of neurological complications due to COVID-19, according to the literature, is up to 82% and is characterized in the acute period by delirium and seizures (34%), fatigue (32%), myalgia (20%), impaired smell or taste, and headache (13 %). Guillain-Barre syndrome is also registered in 10% of cases and stroke in 2% of cases [1]. Complications can occur with a frequency of more than 33% within six months after COVID-19. According to a published study, in patients treated in the intensive care unit, the frequency of damage to the nervous system was 46% and was characterized by ischemic stroke, dementia, intracranial hemorrhages, parkinsonism, psychotic and anxiety disorders [2]. For the purpose of in-depth diagnosis of neurological manifestations, neuron-specific proteins are widely used as markers of damage to the nervous system. Disruption of the blood-brain barrier (BB) in response to brain injury can increase the concentration of specific molecules in the circulation, and the assessment of the concentration of these molecules in the serum can contribute to the diagnosis of the lesion. Astrocyte-specific proteins such as S100 β are found at high levels in the brain, and their presence in the blood may indicate a loss of blood-brain barrier function or the presence of trauma. Several neuron-specific proteins, including neuron-specific enolase (NSE), are released from damaged neurons and enter the

bloodstream if the integrity of the blood-brain barrier is damaged. The presence of serum albumin, which is detected at high levels in the blood, in the cerebrospinal fluid also indicates damage to the blood-brain barrier [3]. In a study by Antonio Aceti et al. (2020), serum S100b concentration correlated with disease severity as indicated by clinical and laboratory parameters. Researchers have shown correlations of S100b with indicators of distress, including non-neuronal indicators such as ALT, D-dimer, and platelets [4]. In other studies, scientists also suggest the marker S100b and NSE as reliable predictors of clinical severity. The serum S100b level showed significantly higher mean values in the cohorts with severe COVID-19 than in the group with mild symptoms. In a similar pilot study, researchers found an increased level of NSE in blood serum in a group of patients with a severe course than in a group without complications ($p = 0.034$) [5,6].

The purpose of the study was to determine the level of S100b and NSE proteins in children with COVID-19 and to investigate the correlation of indicators with the severity of COVID-19.

Research materials and methods. We conducted a retrospective cohort, observational study. We examined 88 children aged 1 month to 17 years with laboratory-confirmed COVID-19 who underwent inpatient treatment at the Kyiv City Children's Clinical Infectious Disease Hospital in 2021-2022. Children were divided according to the course of the disease into two groups - the control group, which had a complicated course of COVID-19, and the main one without complications. We also made a division by age groups - from birth to 12 months, from 1 to 6 years, from 6 to 10 years and from 10 to 17 years. The main laboratory indicators, data of anamnesis and objective examination were taken into account. During the complex routine examination of the patients during the first day of their stay in the hospital, blood serum of the patients was collected for the purpose of its further examination for the level of neurobiomarkers s100b and NSE by enzyme immunoassay. Fujirebio's "CanAg S100 EIA kit" and "CanAg NSE EIA kit" with a working measurement range of 1--3500 ng/L for s100 and 1-150 $\mu\text{g/L}$ for NSE were used. The study was approved by the bioethical committee of the hospital and informed consent was obtained from the patients. For statistical processing of the results, we used the biostatistical package Statistical software EZR v. 1.54 and performed interval estimation of the distribution, multiple comparisons, and calculated the Pearson and Dunn correlation coefficient.

The results. The range of reference values for S100 is less than 105 ng/L and for NSE $<10 \mu\text{g/L}$, but kit manufacturers emphasize that all values are individual and should be evaluated in conjunction with other laboratory and instrumental data.

According to the results of the cohort study, a predominance of the youngest age group of patients from birth to 12 months was revealed, 42 (47.8%) patients, $p < 0.001$. In the gender structure, boys (56.8%) prevailed over girls (43.2%), $p = 0.071$. Depending on the severity of the condition and the presence of complications, children were divided into two groups, an uncomplicated course was observed in 58 (65%) patients, and a severe course of COVID-19 with complications - in 30 (34%) children. Complications were confirmed by the data of instrumental and laboratory examinations and were represented by interstitial pneumonia accompanied by respiratory failure of

the 1st-2nd stage, bronchopneumonia, acute stenotic laryngotracheitis and purulent tubotitis.

We performed calculations of interval evaluation of NSE and S100 markers in patients with COVID-19 of the main and control groups. The results are presented in Table 1

Table 1.

Interval assessment of neurobiomarkers NSE and S100 in patients with COVID-19

Indicators	Group	Me±m	I quartile	III quartile	Left (95% CI)	Right (95% CI)
NSE	Main	13,27±1,4	8	18,6	9,5	15,31
	Control	15,9±1,4	9,21	22,2	11,04	20,9
S100b	Main	161,2±7,6	133,7	180,8	148	173,14
	Control	168,2±7,6	148,3	190,8	158,03	179

According to the calculations, in the patients of the main group, NSE was observed at the level of 13.269 ± 1.422 , while in the children of the control group, the indicator was higher, it was 15.964 ± 1.404 . Regarding the S100b marker, in children with uncomplicated COVID-19, the marker was 161.202 ± 7.563 , while in control children with more severe disease, S100b was at the level of 168.184 ± 7.588 . These data are also demonstrated in Figures 1 and 2.

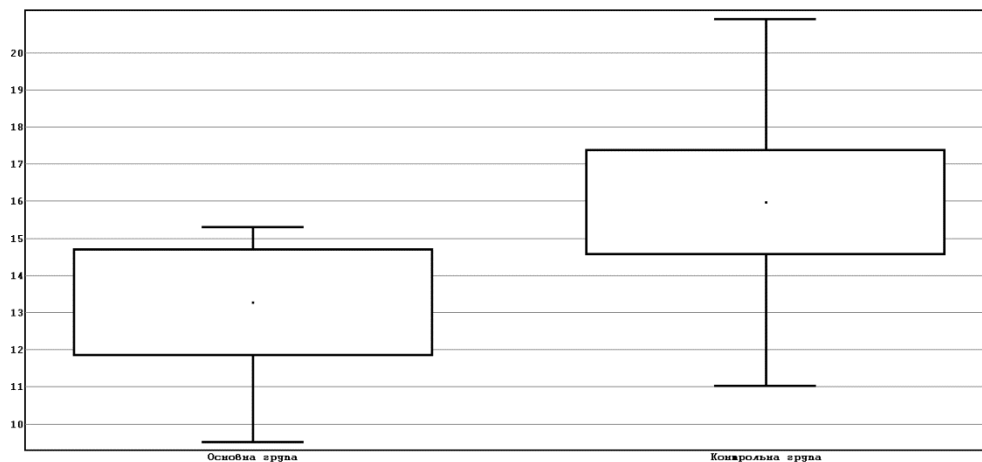


Figure 1. Interval estimation of the mean value of the level of NSE in the blood serum of children with COVID-19 (median, error of the mean, 95% CI are indicated).

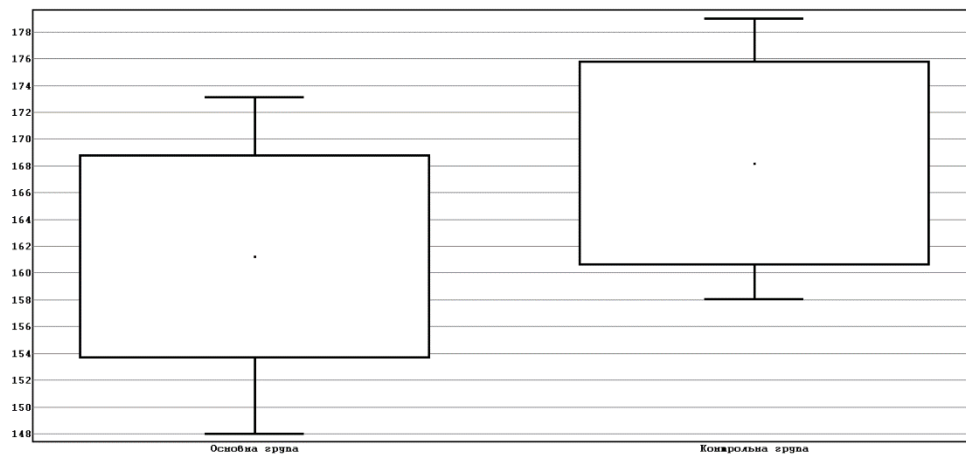


Figure 2. Interval estimation of the mean value of the level of S100b in the blood serum of children with COVID-19 (median, error of the mean, 95% CI are indicated).

According to the interval assessment of the level of biomarkers, patients of the control group had higher NSE and S100b values than patients of the main group.

We also conducted a study of the correlation of the level of biomarkers with the age of patients and laboratory indicators. The results are shown in Figures 3-5.

According to the results of the statistical analysis, there was no linear correlation between the indicators of neuromarkers with platelets, hemoglobin, leukocytes and fibrinogen, $p > 0.1$.

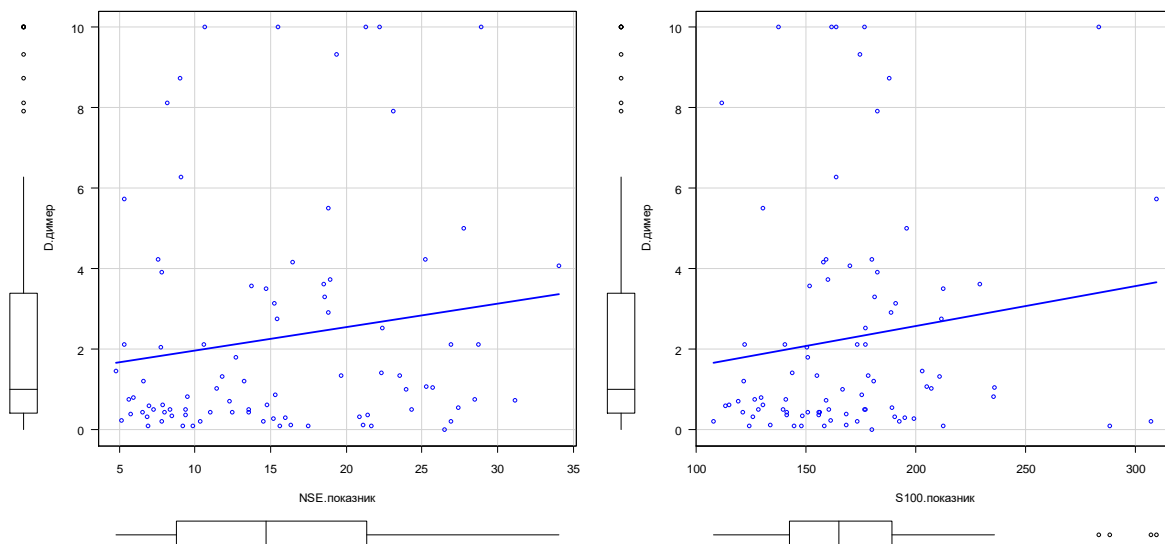


Figure 3. Correlation of NSE and S100b with D-dimer according to the Pearson test.

When conducting a study of the correlation of neurobiomarkers with D-dimer, a linear correlation was found ($p < 0.1$). The value of the correlation coefficient for NSE $r = 0.153$ (95% CI -0.0594-0.352) is statistically significantly different from 0. The value of the correlation coefficient for S100 $r = 0.141$ (95% CI -1-0.311) is statistically significantly different from 0.

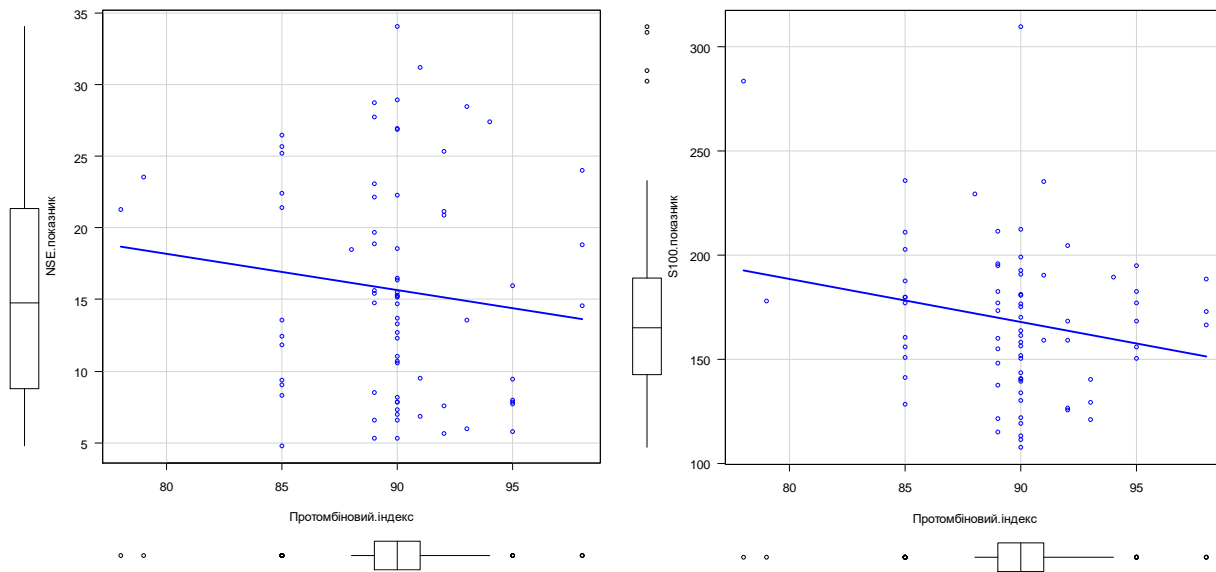


Figure 4. Correlation of NSE and S100b with PTI according to the Pearson test.

When conducting a study of the correlation of neurobiomarkers with the prothrombin index (PTI), a negative linear correlation was found ($p=0.03$). An increase in neurobiomarkers was significantly more often observed with a decrease in PTI. The value of the correlation coefficient for NSE $r = -0.12$ (95% CI $-1-0.07$) is statistically significantly different from 0. The value of the correlation coefficient for S100 $r = -0.204$ (95% CI $-1-0.0131$) is statistically significant is different from 0.

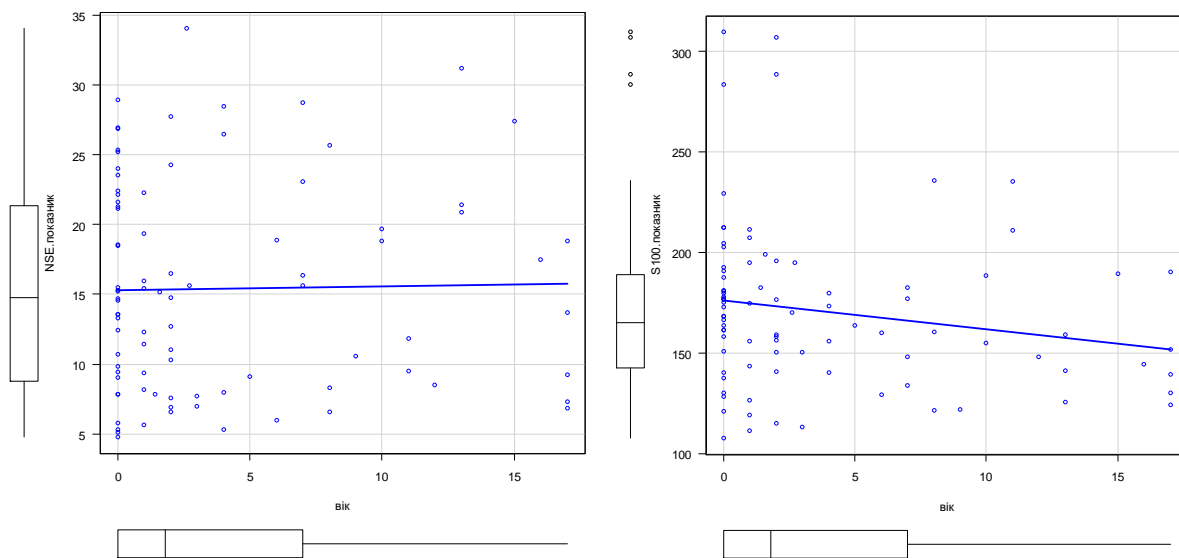


Figure 5. Correlation of NSE and S100b with the age of patients according to the Pearson test.

No correlation was found with the age of the NSE indicator according to the Pearson test ($p=0.57$). However, for the indicator s100b, the relationship was found. An increase in S100 protein was significantly more common in younger patients ($p=0.04$). The value of the correlation coefficient $r = -0.184$ (95% CI $-1-0.0077$) is statistically significantly different from 0.

Conclusions. A correlation between neurobiomarkers and the severity of COVID-19 was revealed. Higher indicators were noted in the group of patients with a

complicated course of the disease. A trend towards a higher level of S100 protein at a younger age of patients ($p=0.04$) was revealed, as well as a linear relationship of neuromarkers with PTI ($p=0.03$) and D-dimer ($p<0.1$).

NSE and S100b proteins are promising neurobiomarkers that may be useful in the diagnosis of COVID-19. Further research into the role of these markers will help predict long-term neurological outcomes and likely complications of COVID-19.

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