The value of the S100β marker in patients with COVID-19

Abstract. Background. The purpose of the work was to determine the level of S100β protein in children with COVID-19 and to investigate the correlation of this neurobiomarker with the severity of COVID-19 and the age of the patients. Materials and methods. We conducted a retrospective, cohort, observational, post-registration 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 Diseases Hospital (Kyiv, Ukraine) 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 group without complications. We also made a division by age groups: 0–12 months, 1–6, 6–10 and 10–17 years. The main laboratory indicators, data of anamnesis and objective examination were taken into account. During the comprehensive routine examination of the patients on the first day of their stay in the hospital, the blood serum was collected for further examination for the level of S100β neurobiomarker by enzyme immunoassay. CanAg S100 EIA kit (Fujirebio) with a working measurement range of 1–3500 ng/L for S100β marker was used. The research was carried out in accordance with the Declaration of Helsinki principles. The research protocol was approved by the Local Ethics Committee of the institution mentioned in the work. Informed consent of parents and children was obtained. In the study, we used statistical research, analytical methods, and the method of empirical research. Results. When comparing the main and control groups by age, the age of patients from birth to 12 months was a significant indicator (44.8 % in the main group vs. 23.3 % in controls), p = 0.049. When conducting a study on S100β correlation with D-dimer, a linear correlation was found (r = 0.141; 95% CI –1…0.311; p < 0.1), as well as a negative linear correlation with prothrombin index (r = –0.204; 95% CI –1…0.0131; p = 0.03) and age (r = –0.184; 95% CI –1…0.0077; p = 0.04). Conclusions. A correlation between S100β neurobiomarker, age and severity of COVID-19 was revealed. Higher indicators were noted in the group of patients with a complicated course of the disease. A tendency towards a higher level of protein S100β at a younger age was revealed, as well as a linear relationship of neuromarkers with prothrombin index and D-dimer.

Keywords: coronavirus infection; COVID-19; neuromarker; S100β; biomarker; children.

Introduction

The long course of the pandemic, the frequency of deaths and complications of the coronavirus infection (COVID-19) have become a real challenge for scientists in finding approaches to timely diagnosis and avoiding possible consequences. As of the beginning of February 2023, there are 672 million laboratory-confirmed cases of COVID-19 worldwide, of which 6.85 million have been fatal. In Ukraine during this period, there were 5.68 million laboratory-confirmed cases and 119 thousand — fatal [1].

In the structure of COVID-19, the incidence in children occupies a significant place and with the years of the pandemic, the frequency of their morbidity continues to grow. It was found that in young children, COVID-19 begins acutely (90.0 %) with intoxication (75.0 %), fever (65.0 %), nasal congestion (25.0 %), rinocongregation (20.0 %), dry cough (60.0 %), increased erythrocyte sedimentation rate (ESR) and C-reactive protein (55.0 %). In older children, the disease is manifested by fever (73.33 %), pharyngitis (66.67 %), dry cough (73.33 %), anosmia (20.0 %), leukopenia (20.0 %), acceleration of ESR (20.0 %) and reduction of prothrombin (13.33 %) without pulmonary lesions (73.33 %) [2].

Damage to the nervous system is one of the most frequent complications of the disease. The frequency of neurological complications due to COVID-19, according to the
literature, varies from 15 to 80 % in adults and children and is characterized in the acute period by delirium and seizures (34 %), fatigue (32 %), myalgia (20 %), impaired smell or taste and headache (13 %). Guillain-Barré syndrome is also registered in 10 % of cases and stroke in 2 % of cases [3, 4]. Published meta-analyses indicate that 16.7 % of children with COVID-19 have nonspecific neurological symptoms such as headache, myalgia, fatigue, and specific neurological deficits occur in 1 % of pediatric patients in the form of encephalopathies, seizures, or meningeal signs [5, 6]. Multisystem inflammatory syndrome, considered a post-infectious hyperinflammatory state associated with SARS-CoV-2 infection, is also associated with a high incidence of neurological involvement, ranging from 12 to 50 % depending on disease severity and including status epilepticus, focal deficit, headache, hallucinations and encephalopathy [7–9].

For the purpose of in-depth diagnosis of neurological manifestations, neuron-specific proteins are widely used as markers of damage to the nervous system. An example of such proteins are S100 proteins. This is a group of specific calcium-binding proteins of astrocytic neuroglia. Their concentration in the brain is almost 90 % of all soluble protein fractions of nerve cells. About 90 % of S100 proteins are contained in astrocytes, 10 % in neurons, and a minimal amount in oligodendrocytes. In cells, they are localized mainly in the cytoplasm, as well as in the synaptic membrane and chromatin. To date, 25 S100 complexes have been described, which include 16 S100A proteins (S100A1-S100A16) as well as others (e.g., S100B, S100G, S100P, and S100Z) [10]. Among S100B, two types of proteins are distinguished — S100 (ββ) and S100 (αβ), which are present in high concentration in glial cells and neurolemmocytes. They are one of the leading molecular components of intracellular systems that ensure functional homeostasis of brain cells and are regulators of extracellular signals [11].

Numerous published studies indicate an extremely wide range of applications of S100 marker. Determining the level of biomarkers in plasma and assessing the state of the coagulation system is one of the methods for differentiating stroke subtypes [12, 13]; S100β can help in the early assessment of the risk of cerebral ischemia in patients. In the study of Y. Tanaka et al. (2009), a correlation was found between the level of S100β in the plasma, hematoma volume, and the severity of brain edema after simulating intracranial hemorrhage against the background of hypertensive disease [14]. In the pathology of the central nervous system, the level of S100β positively correlates with the degree of dysfunction of the blood-brain barrier (BBB). In case of subarachnoid hemorrhage, the level of S100β in the cerebrospinal fluid was significantly increased [15]. The value of biomarkers of brain damage is also used to predict the consequences of brain injury [16, 17]. In patients with depression, studies have shown increased levels of S100β in serum and cerebrospinal fluid [18–20]. Changes in the level of the biomarker were also detected in Alzheimer’s disease [15, 21], migraine [22] and schizophrenia [23]. In experimental studies, an increase in the concentration of S100β was also found in the presence of intracranial oligodendroglioma, pituitary adenoma and neuroblastoma [24, 25]. Serial studies of the protein allow monitoring the effectiveness of treatment and detecting relapses in the early stages [25, 26].

An increase in the level of S100β is detected in newborns with asphyxia, ischemic brain damage, ischemia-reperfusion syndrome, perfusion, in pregnant women in amniotic fluid with an increased risk of fetus hypoxia. The protein content test is used to diagnose perinatal brain damage in newborns of different gestational ages. In children of 48–72 h of life, elevated S100 is an unfavorable prognostic factor. An increase in the level of S100β was observed in children with neuroinfections, in particular, encephalitis [27, 28].

In addition, the researchers found a correlation of the biomarker level with the severity of bilirubin-induced neurotoxic encephalopathy in newborns. A significantly increased level of S100β was detected in newborns with pathological changes in the encephalogram and neurological disorders [29].

Abnormalities of BBB in response to brain damage can increase the concentration of specific molecules in the blood 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 S100β are found in high amounts in the brain, and their presence in the blood may indicate loss of BBB function or injury. The presence of serum albumin, which is detected at high levels in the blood, in the cerebrospinal fluid also indicates a lesion of the BBB [30].

In addition, there are studies that describe the development of lung pathologies in case of dysregulation of the responses of certain types of S100 proteins. In particular, the authors mentioned such diseases as asthma, chronic obstructive pulmonary disease, idiopathic pulmonary fibrosis, cystic fibrosis, pulmonary hypertension and lung cancer, described their mechanism of occurrence and determined the role of proteins in the pathogenesis [31].

With the emergence of the new SARS-CoV-2 and the spread of the pandemic, scientists began to study the role of S100 proteins in the development of COVID-19. In preclinical animal models and patients infected with SARS-CoV-2, elevated levels of immature neutrophils and dramatically increased levels of S100 proteins were observed. In addition, multivariate analysis of patient samples demonstrated that elevated S100 levels were independently associated with mortality [32]. Serum S100 levels in patients with COVID-19 have been associated with severity and increased inhospital mortality [33] and an early indicator of respiratory failure [34].

Considering the lack of similar local studies of this marker in COVID-19, we decided to evaluate the level of S100β protein in children with COVID-19 and determine the presence of relationships.

The purpose of the work was to determine the level of S100β protein in children with COVID-19 and to investigate the correlation of this neurobiomarker with the severity of COVID-19 and the age of the patients.

Materials and methods

We conducted a retrospective, cohort, observational, post-registration 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 Diseases Hospital (Kyiv, Ukraine) in 2021–2022. Children were divided into two groups according to the course of the disease: the control group, which had a complicated course of COVID-19, and the main group without complications. We also made a division by age groups: 0–12 months, 1–6, 6–10 and 10–17 years. The main laboratory indicators, data of anamnesis and objective examination were taken into account. During the comprehensive routine examination of the patients at the first day of their stay in the hospital, the blood serum was collected for the purpose of its further examination for the level of S100β neurobiomarker by enzyme immunoassay. CanAg S100 EIA kit (Fujirebio) with a working measurement range of 1–3500 ng/L for S100β marker was used. The research was carried out in accordance with the Declaration of Helsinki principles. The research protocol was approved by the Local Ethics Committee of the institution mentioned in the work. Informed consent of parents and children was obtained for conducting research. In the study, we used statistical research, analytical methods, and the method of empirical research.

To perform statistical calculations, we used the statistical software EZR v. 1.54 package using methods of descriptive statistics. The median (Me) 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, and calculated the Pearson correlation coefficient.

**Results**

Table 1 shows the age and sex indicators of the study groups.

According to the results of the cohort study (Table 1), no significant difference was found in the gender of the children between the main and control groups (p > 0.18), however, boys predominated in the structure of both groups. Regarding age, the main group consisted mostly of patients under 12 months: 26 (44.8 %), p < 0.001. In the control group, children of 1–6 years prevailed, accounting for 12 (40 %) cases, p < 0.001. The age category of children from 6 to 10 years was the least numerous in both groups: 5 (8.6 %) patients in the main group and 5 (16.7 %) in the control one. When comparing the main and control groups, the age of patients from birth to 12 months was a significant indicator (44.8 % in the main versus 23.3 % in the control group), p = 0.049. Children were divided into two groups depending on the severity of condition and the presence of complications: an uncomplicated course was observed in 58 (65 %), and a severe COVID-19 with complications — in 30 (34 %) cases, p < 0.001. Detailed characteristics are shown in Fig. 1.

Complications were confirmed by the data of instrumental and laboratory examinations and were represented by interstitial pneumonia in 22 (73.4 %) cases, which was accompanied by respiratory failure stage 1–2, bronchopneumonia in 1 (3.3 %), acute stenotic laryngotracheitis in 6 (20 %) and purulent tubootitis in 1 (3.3 %) patient (Fig. 1).

When conducting a statistical analysis, we calculated S100β intervals in patients with COVID-19 of the main and control groups. The results are presented in Table 2.

The range of reference values for S100β is less than 105 ng/L, but the manufacturers of this kit emphasize that all values are individual and should be considered in conjunction with other laboratory and instrumental indicators.

According to the calculations, in the patients of the main group, S100β was observed at the level of 161.2 ± 7.6, while

**Table 1. Age and gender characteristics of the groups, abs. (%)**

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Main group</th>
<th>Control group</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>0–12 months</td>
<td>26 (44.8)</td>
<td>7 (23.3)</td>
<td>0.049</td>
</tr>
<tr>
<td>1–6 years</td>
<td>20 (34.5)</td>
<td>12 (40)</td>
<td>0.6</td>
</tr>
<tr>
<td>6–10 years</td>
<td>5 (8.6)</td>
<td>5 (16.7)</td>
<td>0.26</td>
</tr>
<tr>
<td>10–17 years</td>
<td>7 (12.1)</td>
<td>6 (20)</td>
<td>0.32</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Boys</td>
<td>30 (52)</td>
<td>20 (67)</td>
<td>0.18</td>
</tr>
<tr>
<td>Girls</td>
<td>28 (48)</td>
<td>10 (33)</td>
<td>0.18</td>
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</table>

**Figure 1. Characteristics of the complicated course in children of the control group**
in the children of the control group, the indicator was higher, 168.2 ± 7.6. These data are also demonstrated in Fig. 2.

According to the interval assessment of the biomarker level, higher S100β values were observed in patients of the control group than in patients of the main group (Fig. 2).

We also conducted a study on the correlation of S100β biomarker level with the age of patients and some laboratory parameters. The results are shown in Fig. 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.

When conducting a study on the correlation of S100β neurobiomarker with D-dimer (Fig. 3), a linear correlation was found (p < 0.1). The value of the correlation coefficient for S100 r = 0.141 (95% CI −1... 0.311) is statistically significantly different from 0.

A negative linear correlation was found (p = 0.03) between S100β neurobiomarker and the prothrombin index (PTI) (Fig. 4). An increase in S100β indicator was significantly more often observed with a decrease in PTI. The value of the correlation coefficient for S100β r = −0.204 (95% CI −1... 0.0131) is statistically significantly different from 0.

Correlation with the age of S100β indicator (Fig. 5) according to the Pearson test was found (p = 0.04). Elevation of the studied protein was significantly more common in younger patients. The value of the correlation coefficient r = −0.184 (95% CI −1... 0.0077) is statistically significantly different from 0.

**Discussion**

S100 is a large family of small-molecule calcium-binding proteins composed of numerous isoforms with structural similarities and functional differences. This protein was named S100 in 1965 by the researcher Moore B.W. due to its solubility in 100% ammonium sulfate at neutral pH [35].

As for today, S100 protein is quite common in the diagnosis of cancer, so in most domestic laboratories, it is presented precisely as a tumor marker. However, foreign literary sources indicate the detection of this protein in case of damage to the nervous system [12–14, 16, 17, 36].

Singh P. and Ali S.A. (2022) described the multifunctional role of the S100 protein family in the human immune system. Researchers found that S100 isoforms serve as an alarm signal, an antimicrobial peptide, a proinflammatory

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Group</th>
<th>Me ± SD</th>
<th>I quartile</th>
<th>III quartile</th>
<th>Left (95% CI)</th>
<th>Right (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S100β</td>
<td>Main group</td>
<td>161.2 ± 7.6</td>
<td>133.7</td>
<td>180.8</td>
<td>148</td>
<td>173.14</td>
</tr>
<tr>
<td></td>
<td>Control group</td>
<td>168.2 ± 7.6</td>
<td>148.3</td>
<td>190.8</td>
<td>158.03</td>
<td>179</td>
</tr>
</tbody>
</table>
stimulator, a chemoattractant, and a metal scavenger during the innate immune response. Therefore, they are of crucial importance in the treatment of autoimmune diseases. In their work, the authors presented and described a number of unique characteristics of S100 protein: the ability to control immunological processes, regulate inflammation, the ability of the extracellular protein S100 to regulate cell death, differentiation, proliferation and migration in different types of cells, and possessing antimicrobial properties. The researchers also claim that elevated levels of S100β are associated with a severe course of COVID-19 and suggest its use as a potential biomarker for predicting severity in patients with COVID-19 [37]. This statement correlates with our study, as we also observed higher levels of S100β protein in children with severe COVID-19.

A study by Bagheri-Hosseinabadi Z. et al. (2022) also analyzed the prognostic value of S100 protein in predicting severity of COVID-19. The authors found a positive correlation with D-dimer and confirmed a significant increase in proteins with a severe course of COVID-19 compared to patients with a mild course of the disease [38]. Likewise, in the publication by Antonio Aceti et al. (2020), the concentration of S100β in blood serum correlated with the severity of COVID-19, as indicated by clinical and laboratory parameters. The researchers pointed to correlations of S100β with indicators of distress, including ALT, D-dimer, prothrombin index, platelets, and inflammatory markers procalditin and C-reactive protein [39]. In other studies, scientists also suggest S100β marker as a reliable predictor of clinical severity. Serum S100β showed significantly higher mean values in the cohorts with severe COVID-19 than in the mildly symptomatic group [40].

Conclusions

A correlation between neurobiomarker S100β, age and 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 correlation of neuromarkers with PTI (p = 0.03) and D-dimer (p < 0.1).

S100β protein is a promising neurobiomarker that may be useful in the diagnosis of COVID-19. Conducting further studies on the role of this marker will help predict the long-term neurological consequences and possible complications of COVID-19.

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Information about author
Iryna Seriakova, Assistant at the Department of Pediatric Infectious Diseases, Bogomolets National Medical University, Kyiv, Ukraine; e-mail: ikvaluikiv@ukr.net; https://orcid.org/0000-0002-2793-6584


Значення маркера S100β у дітей із COVID-19

Резюме. Мета: визначити рівень протеїну S100β у дітей із COVID-19 і дослідити кореляційний зв’язок цього нейробіомаркера з тяжкістю COVID-19 та віком пацієнтів. Матеріали та методи. Проведено ретроспективне когортне обережнє постреструктуріювання дослідження. Обстежено 88 дітей віком від 3 місяців до 17 років із лабораторним підтвердженням COVID-19, які проходили стаціонарне лікування в КНП «Київська міська дитяча клінічна інфекційна лікарня» у 2021–2022 рр. За пе-ребігом захворювання пацієнтів розділили на дві групи: контрольну, у якій спостерігався неукладений перебіг COVID-19, та основну групу без ускладнень. Також ми провели розподіл за віковими групами: 0–12 місяців, 1–6, 6–10 та 10–17 років. Враховували основні лабораторні показники, дані анамнезу та об’єктивного обстеження. Під час комплексного рутинного обстеження хворих протягом першої доби перебування в стаціонарі була зібрана сироватка крові з метою її подальшого дослідження щодо рівня нейробіомаркера S100β методом імуноферментного аналізу. Застосовували набір CanAg S100 EIA kit компанії Fujirebio з робочим діапазоном вимірювань 1–3500 нг/л для маркера S100β. Дослідження відбулися відповідно до принципів Гельсінської декларації. Протокол дослідження ухвалено локальним етичним комітетом зазначеніо в роботі установи. Отримано інформовану згоду батьків та ді-тей. Під час роботи були використані статистичні й аналітичні методи, метод емпіричного дослідження. Результати. При порівнянні основної та контрольної груп за віком значним показником виявився вік пацієнтів від народження до 12 мі-сяців (44,8 % в основній групі проти 23,3 % у контрольній), р = 0,049. При проведенні дослідження кореляції нейробіомаркера S100β з D-димером був виявлений лінійний кореля-ційний зв’язок (r = 0,141; 95% ДІ –1... 0,311; р < 0,1), а також негативний лінійний кореляційний зв’язок — з протромбіновим індексом (r = –0,204; 95% ДІ –1... 0,0131; р = 0,03) та віком (r = –0,184; 95% ДІ –1... 0,0077; р = 0,04). Висновки. Ви-явлено кореляційний зв’язок між нейробіомаркером S100β, віком та тяжкістю COVID-19. Вищі показники відмічалися у групи пацієнтів з ускладненим перебігом захворювання. Ви-явлено тенденцію до вищого рівня протеїну S100β при меншо-му віці пацієнтів, а також лінійний зв’язок нейромаркерів із протромбіновим індексом та D-димером.

Ключові слова: коронавірусна інфекція; COVID-19; ней-ромаркер; S100β; біомаркер; діти