ПРОВОСПАЛИТЕЛЬНЫЕ ЦИТОКИНЫ VEGFA, IL-6, IL-8 КАК МАРКЕРЫ ГЕПАТОТОКСИЧНОСТИ ПОСЛЕ COVID-19

Уревский М.А., Ильмухина Л.В., Саранская Я.Е., Лапшин А.А., Гафурова Р.Р.

ФГБОУ ВО «Ульяновский государственный университет», г. Ульяновск, Россия

Резюме. Механизм гепатоцелюллярного повреждения печени после COVID-19 – многофакторный процесс. В литературе наиболее обсуждаемыми причинами являются прямые цитолитические повреждения печени вследствие воспалительной реакции после COVID-19, лекарственно индуцированной гепатотоксичности и прямого цитотоксического действия вируса. Существуют наблюдения, что инфекция SARS-CoV-2 вызывает реактивацию вируса гепатита В, однако взаимодействие между вирусом гепатита С и SARS-CoV-2 описано мало. Течение коронавирусной инфекции связано с выраженной экспрессией провоспалительных цитокинов – участников мультисистемного воспалительного ответа – IL-1β, IL-6, IL-8, IL-18, MCP-1, TNFα, что вносит существенный вклад в наблюдаемые ранние и поздние нарушения функции печени. Цель исследования – оценить роль провоспалительных цитокинов (VEGFA, IL-8, IL-6, MCP-1, TNFα, IL-18) как дополнительных маркеров гепатотоксичности после COVID-19. Исследование выполнено в период с марта по август 2022 года на базе поликлиики № 2 ЦК МСЧ г. Ульяновска. Пациенты были разделены на 2 группы: 1-я группа – с повышением аминотрансфераз на фоне лечения от COVID-19 и/или в последующие 3-6 месяцев после перенесенного заболевания без вирусного поражения печени (n = 42), 2-я группа – пациенты с ко-инфекцией (хроническим вирусным гепатитом С (HCV) и COVID-19 (n = 26). Методом иммуноферментного анализа оценивались уровни цитокинов – VEGF-A, IL-6, IL-8, MCP-1, IL-18, TNFα в сыворотке крови. Статистический анализ проводился с использованием программы StatTech v.3.1.4. Выявлено сопоставимое повышение уровня трансаминаз и С-реактивного белка в обоих группах, значимо отличающееся от референсных значений. Установлены прямые корреляционные связи умеренной силы (линейная корреляция по Спирмену) между следующими цитокинами: TNFα-MCP-1 (R = 0,559; p = 0,001), TNFα-VEGFA (R = 0,400; p = 0,002), TNFα-IL-6 (R = 0,503; p = 0,001). Нами установлено значимое повышение уровня VEGFA в сыворотке крови пациентов 1-й группы (гепатотоксичность после COVID-19) (Me ($Q_{0,25}$-$Q_{0,75}$): 522 (250-1002), p = 0,001) и у пациентов 2-й группы (HCV + COVID-19) (Me 1196, $Q_{0,25}$-$Q_{0,75}$: (73-432). Аналогичная тенденция наблюдается с сывороточными уровнями IL-6 и IL-8 в 1-й группе пациентов, выражено превышающими значения цитокинов у здоровых доноров и достоверно выше, чем у пациентов 2-й группы. Выявленные корреляционные

Адрес для переписки:

Уревский Марк Алексеевич
ФГБОУ ВО «Ульяновский государственный университет»
432017, Россия, г. Ульяновск, ул. Льва Толстого, 42.
Тел.: 8 (987) 633-01-96.
E-mail: mark.urevskiy@gmail.com

Address for correspondence:

Mark A. Urevskii
Ulyanovsk State University
42 Leo Tolstoy St
Ulyanovsk
432017 Russian Federation
Phone: +7 (987) 633-01-96.
E-mail: mark.urevskiy@gmail.com

Образец цитирования:

М.А. Уревский, Л.В. Ильмухина, Я.Е. Саранская, А.А. Лапшин, Р.Р. Гафурова «Провоспалительные цитокины VEGFA, IL-6, IL-8 как маркеры гепатотоксичности после COVID-19» // Медицинская иммунология, 2023. Т. 25, № 4. С. 803-808.
doi: 10.15789/1563-0625-PCV-2843

For citation:

M.A. Urevskii, L.V. Ilmukhina, Ya.E. Saranskaya, A.A. Lapshin, R.R. Gafurova "Proinflammatory cytokines VEGFA, IL-6, IL-8 as markers of hepatotoxicity after COVID-19", Medical Immunology (Russia)/Meditsinskaya Immunologiya, 2023, Vol. 25, no. 4, pp. 803-808.
doi: 10.15789/1563-0625-PCV-2843

DOI: 10.15789/1563-0625-PCV-2843

связи между воспалительными цитокинами доказывают однонаправленность изменений в функционирований регуляторной сети, контролирующей иммунные вирус-индуцированные реакции.

Ключевые слова: гепатотоксичность, COVID-19, воспалительные маркеры, васкулоэндотелиальный фактор роста, IL-6, IL-8

PROINFLAMMATORY CYTOKINES VEGFA, IL-6, IL-8 AS MARKERS OF HEPATOTOXICITY AFTER COVID-19

Urevskii M.A., Ilmukhina L.V., Saranskaya Ya.E., Lapshin A.A., Gafurova R.R.

Ulyanovsk State University, Ulyanovsk, Russian Federation

Abstract. The mechanism of hepatocellular liver damage after COVID-19 is a multifactorial process. The most widely discussed causes are cytolytic liver damage due to the inflammatory response after COVID-19, drug-induced hepatotoxicity and direct cytotoxic effect of the virus. There are observations that SARS-CoV-2 infection causes hepatitis B virus reactivation, but little has been described about the interaction between hepatitis C virus and SARS-CoV-2. The course of coronavirus infection is associated with marked expression of proinflammatory cytokines, participants in the multisystem inflammatory response, IL-1β, IL-6, IL-8, IL-18, MCP-1, TNFα, which contribute significantly to the observed early and late liver function impairment. The aim of the study was to evaluate the role of proinflammatory cytokines (VEGFA, IL-8, IL-6, MCP-1, TNFα, IL-18) as additional markers of hepatotoxicity after COVID-19. The study was performed between March and August 2022. Patients were divided into 2 groups: Group 1 – with increased aminotransferases against the background of treatment from COVID-19 and/or in the following 3-6 months after the disease without viral liver damage (n = 42), Group 2 – patients with co-infection (chronic viral hepatitis C (HCV) and COVID-19 (n = 26). The levels of cytokines – VEGF-A, IL-6, IL-8, MCP-1, IL-18, TNFα in blood serum were estimated by enzyme immunoassay method. Statistical analysis was performed using StatTech v. 3.1.4. The results of the study revealed a comparable increase in the level of transaminases and C-reactive protein in both groups, significantly different from the reference values. Direct correlations of moderate strength (linear Spearman correlation) were found between the following cytokines: TNFα-MCP-1 (R = 0.559; p = 0.001), TNFα-VEGFA (R = 0.400; p = 0.002), TNFα-IL-6 (R = 0.503; p = 0.001). We diagnosed a significant increase in serum VEGFA levels in group 1 patients (hepatotoxicity after COVID-19) (Me ($Q_{0.25}$-$Q_{0.75}$): 522 (250 to 1002), p = 0.001) and in group 2 patients (HCV + COVID-19) (Me 1196, $Q_{0.25}$-$Q_{0.75}$: (73 to 432). Similar trend with the level of IL-6, IL-8, exceeding the values of cytokines in healthy donors and significantly higher than in group 2 patients. Identified correlations between inflammatory cytokines prove unidirectional changes in the functioning of the regulatory network controlling immune virus-induced reactions.

Keywords: hepatotoxicity, COVID-19, inflammatory markers, vascular endothelial growth factor, IL-6, IL-8

Introduction

Post-COVID syndrome – a condition occurring after COVID-19, caused in particular by multisystem inflammatory reaction in the body, having a variety of pathological symptoms, and in particular hepatotoxic manifestations [9]. One of the key reasons for the development of hepatotoxicity after a new coronavirus infection is direct cytolytic liver damage due to a severe inflammatory reaction after COVID-19, drug-induced damage and direct cytotoxic effect of the virus [1, 6].

Patients with SARS-CoV-2 infection and without previous liver disease showed signs of cytolytic liver damage proportional to the severity of COVID-19. Patients with cirrhosis were at higher risk of developing severe COVID-19 and worse liver-related outcomes compared to patients without cirrhosis [3]. A series of meta-analyses on hepatotoxicity after COVID-19 have been published with preliminary conclusions

about the insufficiency and ambiguity of laboratory and clinical data considering transaminases, albumin, bilirubin levels in the evaluation of liver status in post-COVID syndrome [2, 10, 11]. The following questions remain debatable – should all patients after COVID-19 be routinely screened for chronic liver disease and should COVID-19 patients with chronic hepatitis B continue antiviral therapy started before SARS-CoV-2 infection, what are the risks of inter-drug drug interactions between CG and COVID-19.

The course of SARS-CoV-2 infection is associated with marked expression of proinflammatory cytokines – participants in a multisystem inflammatory response such as IL-1β, IL-6, IL-8, IL-18, MCP-1, TNFα, which probably contribute significantly to the early and late liver function impairments observed. In some patients with severe disease, laboratory studies indicate an unregulated inflammatory response similar to cytokine release syndrome, characterized by plasma leakage, increased vascular permeability, diffuse intravascular clotting, and immunodeficiency. High serum levels of proinflammatory cytokines, particularly interleukin-6, have been found in these patients. There may be signs of secondary hematophagocytic syndrome [4].

The question of the associated toxic effect of the SARS-CoV-2 virus on patients with chronic hepatitis remains poorly studied. Therefore, there is a need to search for new promising prognostic markers of hepatotoxicity, taking into account the existing pathogenetic mechanisms of virus-induced systemic inflammation.

The aim of the study was to investigate the role of proinflammatory cytokines (VEGFA, IL-8, IL-6, MCP-1, TNFα, IL-18) as additional markers of hepatotoxicity after COVID-19.

Materials and methods

The study was performed in the period from March to August 2022 at the Polyclinic No. 2 of the Central Clinical Medical and Sanitary Unit, named after Honored Doctor V.A. Egorov and the Research Medical and Biological Center of Ulyanovsk State University. Criteria for inclusion in the study – the age of the patient from 18 to 70 years, a confirmed case of COVID-19 infection from 3 to 12 months ago; increase in aminotransferases (AST, AST) 2-4 times higher than normal during acute and early long-term periods; having informed consent. The patients were divided into 2 groups: Group 1 – with increased aminotransferases against the background of treatment for COVID-19 and/or in the following 3-6 months after the disease without viral liver damage (n = 42, median age – 52) and Group 2 – patients with co-infection (chronic viral hepatitis (HCV) and COVID-19 (n = 26, median age – 46). Exclusion criteria – no elevation of aminotransferases, age less than 18 years. All patients were determined during the period of follow-up after COVID-19 with levels of AST, ALT, C-reactive protein. Determination of cytokines VEGFA, IL-6, IL-8, MCP-1, IL-18, TNFα in blood serum was performed by ELISA (Vector-Best, Novosibirsk, Russia). Statistical analysis was performed using StatTech v. 3.1.4 (Stattech, Russia). Quantitative indices were assessed for their correspondence to normal distribution using the Kolmogorov-Smirnov criterion. If there was no normal distribution, quantitative data were described using median (Me) and lower and upper quartiles ($Q_{0.25}$ to $Q_{0.75}$). Comparison of the two groups for a quantitative indicator whose distribution differed from normal was performed using the Mann–Whitney U criterion.

Results and discussion

When assessing the general condition of patients by questionnaire at follow-up after COVID-19, it was found that more than 74% of patients included in the study complained of shortness and asthenia, 15% had edema, and 23% had muscle and joint pain. Seventy-three percent were vaccinated against COVID-19. 63% of the patients included in the study had a mild form of the disease.We found that

TABLE 1. SERUM LEVELS OF TRANSAMINASE AND C-REACTIVE PROTEIN IN PATIENTS OF THE STUDIED GROUPS

Group	ALT (U/L)	AST(U/L)	C-reactive protein (mg/L)
1st group	64.3 (46.8-78.1)	54.5 (32.1-75.8)	11.5 (7.5-14.8)
2nd group	84.6 (33.5-130.7)	77.3 (35.5-101.6)*	8.3 (3.6-13.5)

Note. *, the differences in the indicators are statistically significantly different between the groups ($p < 0.05$).

TABLE 2. SERUM LEVELS OF PROINFLAMMATORY CYTOKINES IN PATIENTS OF THE STUDY GROUPS

Indicator	1st group (n = 42)	2nd group (n = 26)	Level of difference (Mann–Whitney, U test)
VEGFA, pg/mL	522 (250-1002)	196 (73-432)*	p = 0.001
IL-6, pg/mL	4.8 (2.3-15.7)	2.9 (1.4-7.5)*	p = 0.043
IL-8, pg/mL	106.8 (25.4-250.0)	42.5 (8.8-115.2)*	p = 0.014
MCP-1, pg/mL	190 (124-352)	151 (119-185)	p = 0.093
TNFα, pg/mL	6.7 (2.5-12.3)	4.2 (3.5-5.2)	p = 0.218
IL-18, pg/mL	393 (257-550)	380 (273-510)	p = 0.668

within 2-10 months after COVID-19, both groups of patients had elevated levels of C-reactive protein and transaminases (Table 1). At the same time, it should be noted that C-reactive protein values were higher in group 1 patients.

We revealed a significant difference in VEGFA, IL-8, and IL-6 levels in serum of group 1 patients (post-COVID, with elevated transaminases) in comparison with group 2 HCV+COVID-19 (Table 2). Such an increase in cytokines indicates a manifestation of a systemic inflammatory response caused by viral infection. It should also be taken into account that Group 1 patients had more comorbid conditions (AH, DM, CHC) than patients in the HCV group. Maximal high levels of VEGFA and IL-8 in the serum of patients persisted for 2-6 months after COVID-19.

The dynamics of transaminase levels in both groups were not linear and were characterized by a wave-like pattern, independent of the period of COVID-19 (Figure 1).

Study of levels of CRP, IL-18, MCP-1, TNFα between the groups showed that there were no statistically significant differences between them, but higher levels of the studied parameters were noted in patients with liver damage after COVID-19.

We established direct correlations of moderate strength (linear Spearman correlation) between the following cytokines: TNFα-MCP-1 (R = 0.559; p = 0.001), TNFα-VEGFA (R = 0.400; p = 0.002), TNFα-IL-6 (R = 0.503; p = 0.001), which proves unidirectional changes of inflammatory markers during development of post-COVID syndrome in hepatotoxicity patients.

Lu X.N. et al. (2021) showed that patients with COVID-19 and HBV infection had a lower risk of severe events, including ICU hospitalization or

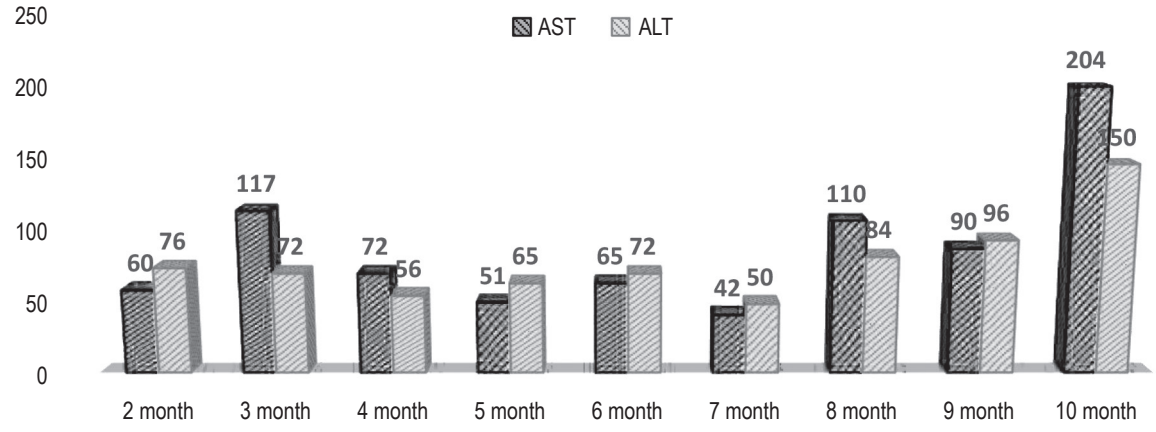

Figure 1. Serum levels of transaminase (U/L) in patients of the studied groups as the time of recovery from COVID-19

death. Factors such as whether patients had other comorbidities, the use of antiviral medications, and the combination of multiple medications may also have influenced patient outcomes [9]. The use of antibodies to block COVID-19-induced cytokine storms that neutralize various cytokines such as IL-1, IFNγ, IL-6 and granulocyte-macrophage colony-stimulating factor (GM-CSF) is an undeniable approach to treating severe patients [7]. However, persistent elevated levels of proinflammatory cytokines after the acute period and their modulation of the multisystem inflammatory response appear to be a leading factor in longCOVID.

Activation of VEGF as an angiogenic factor is a consequence of impaired permeability and local hypoxia caused by infection. "Silent hypoxia" in the lungs of patients in the acute period COVID-19 caused by a combination of biological dysfunctions (microvascular thrombosis, redistribution of capillary blood flow, collapse of the air sac (atelectasis), interstitial edema may be an aggravating factor contributing to systemic complications during rehabilitation [5].

Conclusion

Thus, elevated levels of AST and ALT persisted in some patients after coronavirus infection for 6 months. In patients with hepatotoxicity after COVID-19, we found a statistically significant increase in levels of coagulation mediators – VEGFA, IL-6, IL-8 compared to the HCV + COVID-19 group. The identified correlations between the main cytokines implementing the multisystem inflammatory response in postviral syndrome prove unidirectional changes in the functioning of this regulatory network controlling immune virus-induced responses. Postviral liver dysfunction probably has multifactorial causes, but some of the responses are immune-mediated. The use of additional markers to assess and monitor systemic inflammation, such as – VEGFA, IL-6, IL-8, may be recommended for screening patients at risk of developing liver dysfunction, especially those with comorbid conditions, after COVID-19.

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Авторы:

Уревский М.А. *— студент медицинского факультета Института медицины, экологии и физической культуры, стажер-исследователь Научно-исследовательского технологического института имени С.П. Капицы ФГБОУ ВО «Ульяновский государственный университет», г. Ульяновск, Россия*

Ильмухина Л.В. *— к.м.н., доцент кафедры дерматовенерологии и инфекционных болезней медицинского факультета Института медицины, экологии и физической культуры ФГБОУ ВО «Ульяновский государственный университет», г. Ульяновск, Россия*

Саранская Я.Е. *— старший преподаватель кафедры дерматовенерологии и инфекционных болезней медицинского факультета Института медицины, экологии и физической культуры ФГБОУ ВО «Ульяновский государственный университет», г. Ульяновск, Россия*

Лапшин А.А. *— студент медицинского факультета Института медицины, экологии и физической культуры ФГБОУ ВО «Ульяновский медицины, экологии и физической культуры ФГБОУ ВО «Ульяновский государственный университет», г. Ульяновск, Россия*

Гафурова Р.Р. *— студентка медицинского факультета Института медицины, экологии и физической культуры ФГБОУ ВО «Ульяновский государственный университет», г. Ульяновск, Россия*

Authors:

Urevskii M.A., *Student, Faculty of Medicine, Institute of Medicine, Ecology, and Physical Education, Research Intern at the S. Kapitza Technological Research Institute, Ulyanovsk State University, Ulyanovsk, Russian Federation*

Ilmukhina L.V., *PhD (Medicine), Associate Professor, Department of Dermatovenerology and Infectious Diseases, Faculty of Medicine, Institute of Medicine, Ecology, and Physical Education, Ulyanovsk State University, Ulyanovsk, Russian Federation*

Saranskaya Ya.E., *Senior Lecturer, Department of Dermatovenerology and Infectious Diseases, Faculty of Medicine, Institute of Medicine, Ecology, and Physical Education, Ulyanovsk State University, Ulyanovsk, Russian Federation*

Lapshin A.A., *Student, Faculty of Medicine, Institute of Medicine, Ecology, and a medical student at the Institute of Medicine, Ecology, and Physical Education, Ulyanovsk State University, Ulyanovsk, Russian Federation*

Gafurova R.R., *Student, Faculty of Medicine, Institute of Medicine, Ecology, and a medical student at the Institute of Medicine, Ecology, and Physical Education, Ulyanovsk State University, Ulyanovsk, Russian Federation*

Поступила 16.04.2023
Отправлена на доработку 26.04.2023
Принята к печати 27.04.2023

Received 16.04.2023
Revision received 26.04.2023
Accepted 27.04.2023