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Herpesvirus infections and post-COVID-19 manifestations: a pilot observational study

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Abstract

The global spread of SARS-CoV-2 points to unrivaled mutational variation of the virus, contributing to a variety of post-COVID sequelae in immunocompromised subjects and high mortality. Numerous studies have reported the reactivation of "sluggish" herpes virus infections in COVID-19, which exaggerate the course of the disease and complicate with lasting post-COVID manifestations CMV, EBV, HHV6). This study aimed to describe clinical and laboratory features of post-COVID manifestations accompanied by the reactivation of herpes virus infections (CMV, EBV, HHV6). 88 patients were recruited for this study, including subjects with reactivation of herpes viruses, 68 (72.3%) (main group) and 20 (27.7%) subjects without detectable DNA of herpesviruses (control group): 46 (52.3%) female and 42 (47.7%) male; median age was 41.4 ± 6.7 years. Patients with post-COVID manifestations presented with reactivation of EBV in 42.6%, HHV6 in 25.0%, and EBV plus HHV6 in 32.4%. Compared with controls, patients with herpes virus infections presented with more frequent slight fever temperature, headache, psycho-neurological disorders, pulmonary abnormalities and myalgia ($p < 0.01$), activation of liver enzymes, elevated CRP and D-dimer, and suppressed cellular immune response ($p \leq 0.05$). Preliminary results indicate a likely involvement of reactivated herpes virus infections, primarily EBV infections in severe COVID-19 and the formation of the post-COVID syndrome. Patients with the post-COVID syndrome and reactivation of EBV and HHV6 infections are at high risk of developing various pathologies, including rheumatologic diseases.

Keywords COVID-19 · Herpes virus · Epstein–Barr virus · Rheumatology · An autoimmune disease

Introduction

The current pandemic is caused by the global spread of various strains of SARS-CoV-2. The virus is highly contagious with expressed transmission capacity. However, immune responses to the virus and related clinical manifestations vary widely. Patients with COVID-19 may present with symptoms resembling the common cold, pneumonia, and toxic shock with lethal outcomes [1, 2]. Short and long-term post-acute sequelae of COVID-19 (PASC) are frequently reported. Patients with the post-COVID disease have symptoms similar to systemic autoimmune disorders [3–5].

A systematic review of 57 studies pooling more than 250 000 COVID-19 survivors revealed that more than half of the survivors experienced PASC within 6 months [6]. The most common PASC presents functional mobility impairments, pulmonary problems, and mental health disorders. The long-term PASC may expand health care opportunities, especially in developing countries [1, 6].

The long-term effects of COVID-19 were first described by the British National Institute for Health and Care Excellence (NICE) in agreement with the Scottish Intercollegiate Guidelines Network and The Royal College of General Practitioners in their clinical guidelines dated October 30, 2020 [7]. Moreover, the post-COVID syndrome is now included in the International Classification of Diseases [8].

Variable responses to SARS-CoV-2 have been described in healthy and immunocompromised subjects [2, 4, 6]. The reactivation of the Herpesviridae family viruses may increase the risk of immune suppression with resultant severe COVID-19. The most common immunotropic herpesviruses are Epstein–Barr virus (EBV) and human herpesvirus 6 (HHV 6). These viruses use latency to avoid immune surveillance [9]. The co-infection of SARS-CoV-2 with EBV

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and herpesvirus 1 and 2 (HSV1/2), herpesvirus 6 (HHV6), cytomegalovirus (CMV) may influence the development of severe COVID-19 and post-COVID syndromes.

The study aimed to describe clinical and laboratory features in patients with post-COVID disorders and reactivation of herpes virus infections (CMV, EBV, HHV6).

Materials and methods

The study was conducted in line with the principles of the seventh revision of the Declaration of Helsinki Human Rights (2013), the Council of Europe Convention on Human Rights and Biomedicine, and relevant laws of Ukraine. The Ethics Committee approved the study of Clinical Research at Danylo Halytsky Lviv National Medical University (protocol № 6 dated June 22, 2021). All enrolled subjects signed informed consents.

Participants

Inclusion and exclusion criteria

The inclusion criteria were adults of both sexes, aged 18 to 65 years, indicating COVID-19 transferred during 2019–2020, and existing symptoms that first appeared after COVID-19 and were not explained by alternative diagnoses in the post-COVID period: general lesions of the skin and mucous membranes, nervous system, hepatobiliary system, bone, and joint system, respiratory system, cardiovascular system, urinary system, etc.). Children were excluded from this study, as the focus was adult-onset post-COVID-19. Of the patients identified, we excluded those who had a history of any chronic comorbidities (including systemic autoimmune rheumatic diseases (SARDs)), acute infection of HSV1/2, or acute conditions during treatment pregnancy.

Participants were recruited in Lviv Regional Clinical Hospital, Clinical Hospital of Lviv Railway, and district hospitals of the Lviv region at the request of primary care physicians, therapists, neurologists, pulmonologists, and rheumatologists. Patients who met the criteria for a connection could participate in the study. The Lviv Regional Clinical Hospital (20 patients) was enrolled as the control group. Patients who survived COVID-19 and suffered from symptoms that alternative diagnoses could not be explained in the post-COVID period were initially selected for the study ($n=97$). 9 patients refused to participate in the follow-up study due to manifestations of systemic autoimmune rheumatic diseases (SARDs) and accordingly did not sign an informed consent. The group for further examination included 88 patients: 68 (72.3%) with the post-COVID syndrome and reactivation of herpesviruses (main group) and 20 (27.7%) with a post-COVID syndrome without detectable DNA of herpesviruses (control group). The age of the examined subjects ($n=88$) was 41.4 ± 6.7 years (46 [52.3%] females and 42 [47.7%] males). The main and control groups were age and gender-matched.

Laboratory analysis

The retrospective analysis was based on the outpatient charts data: comprehensive clinical and laboratory exams, complete blood count (CBC), (ALT), aspartate aminotransferase (AST), CRP, D-dimer, ferritin, coagulation tests, glucose level. Molecular genetic studies were performed to detect EBV, HHV6, and CMV. Detection of DNA of EBV, CMV, and HHV6 in peripheral blood, saliva, and oropharynx was performed by PCR using AmpliSens® EBV-screen/monitor-FRT PCR kit with "Rotor-Gene 6000" (Corbett Research, Australia). It was based on amplifying pathogen genome-specific regions using specific EBV primers. To determine the particular class G antibodies to EBV antigens (EBNA-IgG, VCA-IgG), the method of enzyme-linked immunosorbent assay operating test system "Euroimmun" (Germany) was used, according to the manufacturer's instructions.

Statistics

The obtained results were analyzed using the method of variation statistics using the program STATISTICA 10 (Statsoft, USA). The reliability of the difference was assessed by Fisher's test (φ-method). The method can be applied to any particle values, including when the Student's t test is not used, namely when $p<0.25$ and $p>0.75$.

$\varphi = 0.0349 \arcsin \sqrt{p}$ where p–particle, φ – angle in radians.

The probability of particle difference by the method j is determined by

$$F = \left(\varphi_1 - \varphi_2\right)^2 \cdot \frac{n_1 n_2}{n_1 + n_2} \geq F_{st} \left\{ \begin{array}{l} v_1 = 1 \\ v_2 = n_1 + n_2 - 2 \end{array} \right\}$$

where F Fisher's criterion, $n1$, $n2$ are numbers of measurements in groups. The calculated Fisher's criterion is compared with the standard Fisher's criterion for three levels of reliability 0.95, 0.99, 0.999.

Results

A detailed analysis of anamnestic, clinical, and laboratory examinations was performed. According to outpatient charts, it was determined that all patients had a history of COVID-19. Of these, 19 (21.6%) had a mild course of the disease, the other 69 (78.4%) had a moderate and severe

course of the disease, which caused 57 (64.8%) individuals to be hospitalized, including 15 (26.3%)—in the intensive care unit. Pneumonia was diagnosed in 61 (69.3%) individuals (confirmed by radiography, computed tomography, or lung ultrasound). Forty-three patients received non-invasive oxygen therapy, and two were connected to the ventilator. Patients with moderate and severe courses received infusion therapy:

Antiviral, glucocorticosteroids
Antibiotic treatment (protected penicillins, macrolides, cephalosporins of the second or third generation).
Anticoagulants for 7–21 days during the disease.

The reason for appointment 1–4 months after receiving a negative test for SARS-CoV-2 was the presence of post-COVID symptoms: slight fever temperature (76.1%), headaches (77.2%), sleep disorders (96.6%), cognitive impairments (96.6%), constant fatigue (97.7%), irritability (79.5%), depressive thoughts (67.0%), anxiety or indifference (55.7%), persistent cough (44.3%), loss of smell/taste (40.9%), arthralgia (40.9%), myalgia (39.8%), hair loss (36.4%), shortness of breath (36.4%), chest pains (29.5%) (Fig. 1).

According to the results of complex general laboratory tests, CBC revealed increased ESR (from 22 to 36 mm/h) in 34 (38.6%) individuals, lymphopenia in 32 (36.4%) individuals, leukopenia in 19 (21.5%), leukocytosis in 7 (7.95%), thrombocytopenia in 12 (13.6%), thrombocytosis in 15 (17.0%), monocytosis 19 (21.5%), increase in rod-shaped neutrophils in 12 (13.6%) %) patients. Other test results revealed the following: raised ALT in 45 (51.1%) individuals, AST—in 38(43.1%), CRP—in 15 (17.0%), D-dimer—in 34 (38.6%), ferritin—in 16 (18.1%) individuals, changes in coagulogram (increase of fibrin, prothrombin index)—in 14 (15.9%), increased glucose level—in 22 (25.0%) patients.

Since our patients showed signs and symptoms that persisted for more than a month after COVID-19 and were not associated with another diagnosis, we diagnosed post-COVID state—U09.9 (condition after COVID-19, unspecified).

As we have mentioned previously, DNA replication of the virus was not detected according to DNA testing results of CMV, EBV, HHV6 and in saliva, blood, and mucous membrane of the posterior pharyngeal wall of 20 patients. Based on the presence of specific EBNA-IgG and EBV-VCA-IgG in these patients, they had a verified EBV infection, latent phase (control group). In the other 68 patients, EBV and/or HHV6 DNA "+" was detected: EBV DNA—in 29 (42.6%), HHV6 DNA—in 17 (25.0%), EBV and HHV6 DNA—in 22 (32.4%) individuals (main group). Thus, among the main group of patients, EBV reactivation was detected most often—it was detected in 51 (75.0%) individuals, HHV6 reactivation was detected to a lesser extent—in 39 (57.4%) patients. CMV DNA was not detected in any biological media (Figs. 2, 3).

Regarding the media of DNA detection of herpesviruses: most often, the DNA of viruses was detected simultaneously in saliva and mucosa of the posterior pharyngeal wall—in 47 (69.2%) cases, while in 12 (17.6%), it was detected only in the mucosa, in 7 (10.3%) only in saliva, and only EBV DNA was detected in the blood in 2 (2.9%) cases (Fig. 3). These patients were diagnosed with an active phase with chronic EBV /HHV6 infection or co-infection (EBV + HHV6).

From the medical history of the 57 patients previously hospitalized for COVID-19, 51 (89.5%) were in the main group, which was likely more common than the 6 (10.5%)

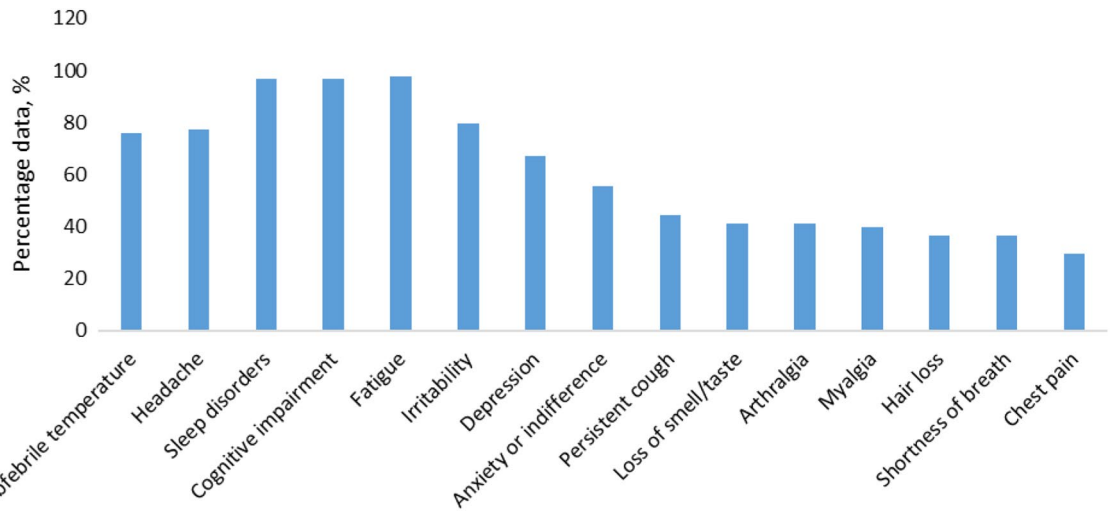

Fig. 1 Frequency of post-COVID-19 symptoms in patients, $n = 88$

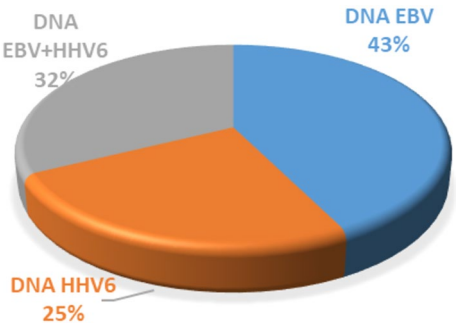

Fig. 2 The prevalence of chronic EBV, HHV6 in the active phase in patients with the post-COVID condition

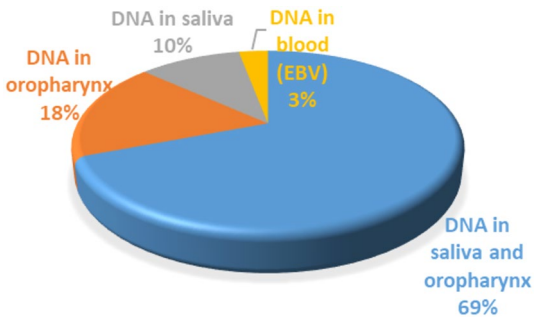

Fig. 3 The prevalence of activated EBV and HHV6 infections depending on biological media

patients in the control group ($p < 0.05$), the majority of patients of the main group were on oxygen therapy, including two who received mechanical ventilation. It should also be noted that pneumonia was diagnosed in 53 (77.9%) individuals of the main group, which was also higher than in the control group—8 (40.0%), $p < 0.05$. Inpatient treatment of the main group of patients lasted longer (16 ± 7 vs. 10 ± 4 days) than the control group. Thus, COVID-19 in patients with reactivation of EBV infection/HHV6 infection or co-infection (EBV + HHV6) was more severe; viral pneumonia was diagnosed more often, and these patients received more intensive treatment extended hospital stay, respectively.

Comparative analysis of clinical features of patients with post-COVID manifestations showed that patients with reactivation of herpesvirus infections were more likely to have sub febrile symptoms, headaches, psycho-neurological disorders, pulmonary abnormalities, and myalgias (Table 1).

It is also noteworthy that general weakness and fatigue were experienced by 94.1% of patients in the main group and 80.0% of the control group.

The comparative analysis of laboratory tests data in the main and control groups was performed. It revealed that elevated ESR, lymphopenia, monocytosis, and increased liver enzymes ALT and AST, CRP, and D-dimer were significantly higher in patients of the main group compared to the control group, $p < 0.05$.

Therefore, clinical and laboratory data features, characterized by more sub febrile temperature, functional joint impairments, pulmonary disorders, mental health problems, activation of liver enzymes, formation of chronic inflammation, and decreased activity of cellular immune response, were revealed in patients with the post-COVID condition. An increased number of patients with myalgias, slight fever temperature, general weakness and fatigue, and prolonged changes in laboratory parameters (lymphopenia, increased acute-phase proteins) may indicate the formation of rheumatic disorders involving the musculoskeletal system.

Table 1 Analysis of clinical manifestations in patients with the post-COVID condition of the main and control groups

Clinical manifestations, amount of cases	Main group, $n = 68$	Control group, $n = 20$	Fisher's criterion, F	Reliability
Slight fever temperature ($n = 67$)	60 (88.2%)	7 (35.0%)	4.620	> 0.99
Headaches ($n = 68$)	59 (86.8%)	9 (45.0%)	3.641	> 0.99
Sleep disorders ($n = 85$)	66 (97.1%)	19 (95.0%)	0.418	< 0.95*
Cognitive impairments ($n = 85$)	68 (100%)	17 (85.0%)	3.127	> 0.99
Irritability ($n = 70$)	60 (88.2%)	10 (50.0%)	3.422	> 0.99
Depressive thoughts ($n = 59$)	51 (75.0%)	8 (40.0%)	2.850	> 0.99
Persistent cough ($n = 39$)	30 (44.1%)	9 (45.0%)	0.070	< 0.95*
Loss of smell/taste ($n = 36$)	27 (39.7%)	9 (45.0%)	0.421	< 0.95*
Arthralgia ($n = 36$)	30 (44.1%)	6 (30.0%)	1.154	< 0.95*
Myalgia ($n = 35$)	31 (45.6%)	4 (20.0%)	2.182	> 0.95
Hair loss	25 (36.7%)	7 (35.0%)	0.145	< 0.95*
Shortness of breath ($n = 32$)	26 (38.2%)	6 (30.0%)	0.684	< 0.95*
Chest pains ($n = 26$)	20 (29.4%)	6 (30.0%)	0.051	< 0.95*

*Difference is not significant

An analysis of medical history and clinical data of patients with chronic EBV infection/HHV6, infection, or co-infection (EBV + HHV6) in the active phase indicated that these disorders were observed to a greater extent in case of reactivation of chronic EBV infection or co-infection of EBV + HHV6 type, which pointed at the potential role of EBV in the formation of post-COVID state and dictated the need for additional antiviral therapy.

Therefore, post-COVID symptoms of the patients in our study occurred against the background of reactivation of herpesviruses, among which the most common was mono EBV infection or co-infection EBV + HHV6. It was determined that COVID-19 in these patients was more severe, with diagnosed pneumonia, which required more intensive treatment. Post-COVID syndrome affected by the reactivation of herpesviruses was accompanied by pronounced functional, psycho-neurological manifestations and changes in laboratory findings, indicating a prolonged inflammatory process with the probable formation of systemic autoimmune disorders.

Discussion

COVID-19 disease is accompanied by a decrease in immunity, which affects the reactivation of chronic infections caused by intracellular pathogens, characterized by lifelong persistence in the human body. Among this group of diseases, the leading role is played by infections caused by herpes viruses, in particular Epstein-Barr virus (EBV) [10]. According to the scientific literature, the level of disease with this virus in children is 50–80%, while it amounts to more than 90% [11]. Reduced general and local immunity in case of any disease, including COVID-19, is a potential trigger for EBV reactivation in the body [12, 13]. According to several authors, co-infection of SARS-CoV-2 with EBV in patients with COVID-19 may contribute to the severity of the clinical manifestations and the duration of the underlying disease or induce the development of the post-COVID condition.

One of the first such studies (January–February 2020) conducted in Wuhan (China) showed that among 67 patients with COVID-19 at Zhenmin Clinic, 37 (55.2%) individuals were seropositive to IgM antibodies of EBV capsid antigen (VCA). Patients with EBV/SARS-CoV-2 co-infection were 3.09 times more likely to develop fever symptoms than patients with SARS-CoV-2 infection alone (95% CI 1.11–8.56; $P = 0.03$). C-reactive protein (CRP) ($P = 0.02$) and AST ($P = 0.04$) were also found to be higher in patients with EBV/SARS-CoV-2 co-infection than in patients with SARS infection alone. EBV/SARS-CoV-2 co-infection was accompanied by fever and symptoms of severe inflammation; such patients required higher doses and duration of corticosteroid therapy than the SARS-CoV-2 infection group alone ($P = 0.03$) [14].

In parallel, a study of 128 patients with COVID-19 admitted to the intensive care unit of Wuhan Hospital № 3 (January 31–March 27, 2020) was performed. Patients were divided into EBV and non-EBV groups depending on whether EBV reactivation was detected. Epidemiological, clinical, and objective data and the results of laboratory studies were analyzed [15]. According to the results, EBV reactivation was detected in 17 (13.3%) patients. In the group with co-infection, the incidence of tachypnea was higher than in the group without EBV ($p < 0.05$); lymphocyte and albumin levels were lower, and serum D-dimer, CRP, and calcium in the EBV group were higher than in the group without EBV, and no significant difference in procalcitonin levels was found between groups. Patients co-infected with EBV/SARS-CoV-2 had more severe clinical manifestations and required more immunosuppressive and oxygen therapy. Mortality rates at 28 and 14 days in the EBV group were significantly higher than those without EBV.

A group of Italian researchers led by Stefania Paolucci and others determined that among the potentially pathogenic viruses (EBV, CMV, HHV6, parvovirus), only EBV was most often discovered. EBV DNA was identified in 40/42 (95.2%) patients with COVID-19 in intensive care units and 51/61 (83.6%) patients from the total number of patients hospitalized with COVID-19. Moreover, the average level of EBV DNA among patients in intensive care units was higher than in general hospitalized patients. In patients with EBV/SARS-CoV-2 co-infection requiring intensive therapy, there was a significant decrease in the number of CD8 and NK T-lymphocytes ($p < 0.05$) and an increase in the number of B-lymphocytes ($p = 0.0172$) in contrast to the patients from the comparison group [16].

Group of A Simonnet et al. examined 34 patients in hospital with confirmed COVID-19 for the presence of EBV, CMV, and HHV-6 DNA in the blood. Viremia with EBV, CMV, and HHV-6 was found in 28 (82%), 5 (15%), and 7 (22%) patients, respectively. EBV reactivation occurred early after reception to the hospital and was associated with a prolonged stay in the hospital [17].

In our study in the group of patients with post-COVID symptoms and active phase EBV and HHV6 infections, most clinical data and laboratory results are similar to those described by other authors. We cannot confirm the exact time of reactivation of herpes virus infections. However, the retrospective data we analyzed during COVID-19 suggest that the reactivation of EBV and HHV6 occurred in the case of SARS CoV-2 infection. Our comparative analysis between groups of patients with post-COVID symptoms also confirms the data of other authors on the role of herpesviruses and, primarily, EBV in the severity of COVID-19. Our data show the high prevalence of activated herpesviruses

(EBV—42.6%, HHV6 DNA—25.0%, EBV and HHV6—32.4%) in patients during the post-COVID period may also indicate their participation in the formation of post-COVID symptoms.

It is described that about 30% of patients with COVID-19 retain symptoms after a period of acute illness [18]. These symptoms include fatigue, cognitive impairment, sleep problems, arthralgia, pharyngitis, myalgia, headaches, fever, gastrointestinal disorders and skin rashes with various manifestations, etc. [19–22]. The combination of these COVID-19-related symptoms has been identified as long-term COVID-19 or post-acute COVID-19 syndrome (PACS) or chronic COVID-syndrome (CCS) [23]. Moreover, long-term COVID-19 may occur in patients who had both mild and severe forms of COVID-19 [19], which coincides with the results of our studies.

Many studies have confirmed that several symptoms associated with long-term COVID-19 are the same or very similar to the symptoms related to EBV reactivation. [24]. In particular, Myalgic Encephalomyelitis/Chronic Fatigue Syndrome is a chronic multisystem disease of unconfirmed etiology. Initial research suggested the potential role of human herpesviruses, especially EBV, in the disease process, but the issues have been controversial for some time. New studies using more advanced approaches have shown that specific proteins encoded by EBV can contribute to immune and neurological abnormalities in subgroups of patients with CFS. In particular, the particular protein EBV deoxyuridine triphosphate nucleotidohydrolase (dUTPase) causes anxiety and pain, exacerbated by chronic stress. dUTPase EBV can change the structure of synapses, neural communication (cognitive impairment), is an inducer of cytokines TNF-α, IL-1β, and IL-6, leading to disruption of the hematoencephalic barrier and penetration of inflammatory mediators, dendritic cells, and T cells into the brain.

Moreover, the ability of dUTPase to act as pathogen-associated molecular patterns (PAMP) is not a unique property only for dUTPase EBV but familiar to herpesviruses (HSV-2, HHV-6A, HHV-8, VZV). It seems likely that reactivation of EBV during COVID-19 is one of the causes of CFS, including in patients with long-term COVID-19 [25]. In our study, many patients in the main group (94.1%) had symptoms of CFS. Cognitive impairment was significantly more common in contrast to patients in the control group (100.0% vs. 85.0%, $p < 0.01$), which confirms the involvement of herpesviruses, especially EBV, in the formation of these disorders in patients with the post-COVID condition.

We also found that EBV (mono EBV—in 42.6%; EBV and HHV6—in 32.4%) was most often identified in patients with the post-COVID state. The data we obtained coincide with the results of other studies. There is evidence that EBV can induce the expression of angiotensin-converting enzyme 2 (ACE2) and the entry of SARS-CoV-2 into epithelial cells [26]. The association of EBV with mitochondrial dysfunction, which may play a key role in susceptibility and recovery from SARS-CoV-2, is described [27]. Moreover, a recent finding is that mitochondrial function is essential for the duration of the antiviral cytotoxic part of T-lymphocytes [22], and EBV infection of T-lymphocytes, respectively, reduces mitochondrial function and inhibits this process [28]. In this regard, it has been observed that patients with SARS-COV-2 have a subset of T-lymphocytes with altered metabolic profiles that are prone to mitochondrial apoptosis and, as a consequence, lymphopenia and an increase in myeloid suppressor cells, which is associated with the severity of the disease and altered expression of anionic channel 1 (VDAC1) – a critical mitochondrial protein [29]. As mentioned above, lymphopenia was also observed in our patients with post-COVID and probably more often in the group affected by chronic EBV or EBV + HHV6 infection in the active phase than in the control group $p \leq 0.05$.

Because both SARS-CoV-2 and EBV can modulate mitochondrial function, an excellent mitochondrial reserve may be vital in determining the severity of COVID-19, as the proper functioning of the immune system depends mainly on the part of this organelle. Authors Nunn, A.V. et al. have suggested co-infection of SARS-CoV-2 and EBV as a double mitochondrial "stroke" that may support the mitochondrial hypothesis underlying the severity of COVID-19 and the long-term symptoms of the disease [30]. As mitochondrial function declines with age, this may be an important factor in why older people are more susceptible to SARS-CoV-2.

New data regularly appear in the modern references, which also confirms the significant role of reactivated herpesviruses and primarily EBV in the formation of post-COVID disorders, which coincides with the results of our work. Indeed, a study by Jeffrey E. Gold et al., consisting of 185 patients with COVID-19, found that the prevalence of long-term symptoms of COVID-19 accounted for 30.3% (56/185), including four asymptomatic patients with COVID-19 [31, 32]. EBV reactivation was detected (based on the determination of early antigen-diffusion IgG (EA-D) or capsid IgM (VCA) EBV antigens) in 66.7% of patients with long-term COVID-19 at the onset of the disease ($p < 0.001$), and in 18 people—21–90 days after a positive COVID-19 test, indicating EBV reactivation shortly after or at the time of COVID-19 infection. The researchers suggested that most of the symptoms of long-term COVID-19 may not be a direct result of the SARS-CoV-2 virus but maybe the development of the reactivation of EBV caused by inflammation of COVID-19.

There are many reports that the symptoms and laboratory data of patients with long COVID-19 are very similar to the symptoms of autoimmune rheumatic disease, mainly systemic lupus erythematosus, rheumatoid arthritis, and others [33–35]: fatigue, myalgia, arthralgia, low-grade fever,

weakness, elevated levels of acute proteins, ESR, changes in general blood tests, etc. The role of EBV in the pathogenesis of autoimmune disorders due to the phenomenon of molecular mimicry and polyclonal activation of B-lymphocytes is also known [3, 35–37]. It is becoming clear that reactivation of herpesviruses and especially EBV, triggered by SARS-CoV-2, can be an actual trigger for the formation of various autoimmune rheumatic diseases. Patients with long COVID-19 need mandatory examination for reactivated herpesvirus infections and medical consultation with rheumatologists.

In our study, symptoms and laboratory abnormalities in patients with post-COVID syndrome and reactivation of herpesviruses resembled symptoms of systemic autoimmune disorders—myalgia, slight fever on the background of general weakness, fatigue, prolonged changes in laboratory parameters (lymphopenia, elevated acute proteins). As mentioned above, eight patients with a complicated previous diagnosis of SARDs were excluded from the study. Therefore, the detected symptoms in the background of laboratory changes may probably indicate systemic autoimmune disorders in these patients including rheumatological. These individuals can be classified as immunocompromised, which requires further observation by rheumatologists and additional specific studies.

Therefore, our data fully confirm the results described by other researchers. Our study will expand the knowledge of rheumatologists about the long-term manifestations of COVID-19 and help differentiate the symptoms of long COVID19 from rheumatic diseases to ensure a timely and accurate diagnosis.

The number of patients included in this study is insufficient for in-depth conclusions. To investigate the possible formation of rheumatic complications in patients with long-COVID and herpes virus infection, we plan further investigations, including studying populations and subpopulations of lymphocytes, and the apoptotic and antiapoptotic activity of NK-cells, cytotoxic cells, ELISA HSV1/2. We plan to study the impact of other herpesviruses, including HSV1/2, on the formation of rheumatic disorders in patients with long-COVID.

Conclusions

In 72.3% of patients with post-COVID condition reactivation of herpes virus infections, EBV and HHV6 were discovered. In these patients, the clinical manifestations have included slight fever temperature, impaired functional activity, myalgia, mental disorders, and pulmonary abnormalities. In patients with the post-COVID condition, some laboratory parameters, such as elevated ESR, lymphopenia, monocytosis, activation of liver enzymes, CRP, and D-dimer, were revealed. Patients with post-COVID conditions and reactivated herpesvirus infections (EBV, HHV6) are at high risk of developing various pathologies, including rheumatologic diseases.

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Declarations

Conflict of interest The authors have no conflict of interest to disclose.

Ethical statement Ethical Committee or Institutional Animal Care and Use Committee Approval: Danylo Halytsky Lviv National Medical University (22/06/2021 protocol № 6).

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