Interruption of viral interference by anti-SARS-CoV-2 vaccination

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Abstract

Epstein-Barr virus (EBV) reactivation may be involved in long-COVID symptoms. Here we evaluated reactivation of parvovirus B19 and several viruses of the herpes family in patients with long-COVID syndrome, how vaccination affected viral interference, and how virus reactivation influenced clinical conditions. Clinical and laboratory data on 252 consecutive patients (97 vaccinated and 155 non-vaccinated) were recorded between April 2021–May 2022 (median 243 days post-COVID-19 infection). Viral IgG and IgM titers were compared between vaccinated or non-vaccinated patients, and age and sex-matched healthy controls. Vaccination was associated with significantly less frequent fatigue and multiorgan symptoms ($P < 0.001$), significantly less cumulative IgM positivity of the investigated viruses, significantly lower plasma levels of IgG subfractions 2 and 4, and significantly lower quantitative Cytomegalovirus (CMV) IgG, CMV IgM, and EBV IgM titers. These results indicate that anti-SARS-CoV2 vaccination interrupts viral crosstalk in patients with long-COVID syndrome. (ClinicalTrials.gov Identifier: NCT05398952)

Introduction

Like other RNA viruses, SARS-CoV-2 can induce a violent cytokine storm, and can potentially cause persistent infections, as evidenced by prolonged viral particle presence in several organs. Persevering viral infection may explain the prolonged symptoms in patients with long-COVID syndrome[1].

Reactivation of certain viruses—such as hepatitis B, Epstein-Barr virus (EBV), cytomegalovirus (CMV), and or herpes simplex virus (HSV)—has been reported among critically ill immunocompetent hospitalized patients, especially in those undergoing immunosuppressive therapy or chemotherapy, reflecting immunosenescence [2,3,4]. Several clinical investigations among patients hospitalized or treated in the intensive care unit (ICU) have confirmed co-infections with SARS-CoV-2 plus other respiratory viruses, such as influenza and respiratory syncytial virus (RSV), or viruses co-localized in the nasopharyngeal area (e.g., EBV and rhinoviruses), suggesting that these other viruses may be reactivated by SARS-COV-2 [5,6,7,8,9,10]. Anecdotal case reports have also confirmed the co-incidence or co-infection of EBV with SARS-CoV-2 in patients with active COVID-19 infection, not requiring hospitalization [11,12,13,14,15]. Some investigations have demonstrated SARS-CoV-2-related EBV reactivation lasting long after the initial COVID-19 illness, suggesting that temporary EBV viremia may play a causative role in the development of chronic fatigue syndrome in the post-acute sequelae or long-COVID phase [16,17,18,19,20,21]. Moreover, studies have reported a reduction of long-COVID symptoms after vaccination [22,23].

In an in vitro study, Verma et al demonstrated that lytic EBV replication enhances the cell surface expression of ACE2 receptors, enabling cellular entry of the SARS-CoV-2 virus, and suggesting DNA–RNA inter-viral communication at the cellular level [24]. Interaction between different viruses, involving competitive inhibition or enhancement of viral replication, has been described in children suffering from respiratory tract infections [25]. However, simultaneous or sequential reactivation of several viruses, and

the behavior of this “viral consortium” after vaccination targeting one single virus (SARS-CoV-2), has not been investigated in patients with previous SARS-CoV-2 infection.

Therefore, in this prospective multicenter study, we aimed to evaluate the SARS-CoV-2-associated reactivation of several DNA viruses of the herpes virus family, including HSV, varicella-zoster virus (VZV), CMV, and EBV, as well as parvovirus B19 in patients diagnosed with long-COVID syndrome during a period when the Delta and Omicron SARS-CoV-2 variants were predominant. We also aimed to assess the effects of vaccination on the symptoms and viral interference, and to investigate how virus reactivation affected clinical conditions and long-COVID syndromes, influenced by immunoprophylaxis against COVID-19 disease.

Results

Between April 2021 and May 2022, a total of 305 patients suffering from persistent long COVID symptoms fulfilling the criteria of long-COVID syndrome [26, 27, 28, 29] were prospectively entered into our registry at the Cardiology Long-COVID Unit of the Division of Cardiology, Department of Internal Medicine II, Medical University of Vienna, Austria (EK: 1008/2021) (ClinicalTrials.gov Identifier: NCT05398952). After the exclusion of 53 patients, 252 patients were included in the current analysis (Fig. 1). Reasons for exclusion were refusal to participate (n = 2), known systemic inflammatory or active malignant diseases (n = 17), newly diagnosed systemic disease (e.g., active hyper- or hypothyroidism, malignant hematologic disorder, acute pulmonary embolism, or ischemic heart disease requiring invasive treatment; n = 21), repeated SARS-CoV-2 infection (n = 7), or incomplete blood sampling for any reason (n = 6). The included patients were divided into two groups: patients who were not vaccinated, and patients who had already received at least two COVID-19 vaccines at the time of their first clinical presentation and blood sampling.

Main study

Table 1 presents the clinical data of the included patients (n = 252). The results show a significant difference in plasma anti-spike protein titer between patients with versus without vaccination.

Table 1
Clinical data of the included patients.

Clinical data	All patients (*n* = 252)	Patients without vaccination (*n* = 97; 38%)	Patients already vaccinated before first clinical presentation (*n* = 155; 62%)	p between w/wo vaccine
Gender female	170 (67.5%)	66 (68.0%)	104 (67.1%)	
Age	43.7 ± 14.2	43.3 ± 13.1	43.9 ± 14.9	
DM	7 (2.8%)	1 (1.0%)	6 (3.9%)	
Hypertension	70 (27.8%)	22 (22.7%)	48 (31.0%)	
HLP	59 (23.4%)	20 (20.6%)	39 (25.2%)	
Smoking	22 (8.7%)	6 (6.2%)	16 (10.3%)	
Syst RR	131 ± 17	130 ± 16	132 ± 17	
RR Diast	84 ± 11	84 ± 10	83 ± 11	
Heart rate (bpm)	72 ± 12	71 ± 11	72 ± 12	
Patient category				
1 (Neuro)	99 (39.3%)	38 (38%)	61 (39%)	
2 (Pulmo)	54 (21.4%)	21 (22%)	38 (25%)	
3 (Cardio)	94 (37.3%)	38 (39%)	56 (36%)	
COVID-related data				
Time between COVID-19 positivity and first clinical presentation (days) (median; IQR)	243 (139;360)	190 (130;275)	278 (143;388)	**0.001**
Time between COVID vaccine and first clinical presentation (days)			173 (77;300)	
Anti-spike protein titer	2272 (133;2500)	137 (35;623)	2500 (2500;2500)	< **0.001**
ECG				
Any ECG Abnormalities	61 (24.2%)	19 (19.6%)	42 (27.1%)	
Rhythm disturbances	14 (5.6%)	6 (6.2%)	8 (5.2%)	
Conduction abnormalities	52 (20.6%)	14 (14.4%)	38 (24.5%)	
QRS duration (ms)	92 ± 14 ms	94 ± 15	91 ± 14	

Data are presented as mean ± SD, median (interquartile range), or number (frequency).

The most common symptoms were neuropsychiatric (without objective pulmonary or cardiovascular abnormalities), followed by dominantly cardiovascular symptoms and pulmonary diseases (Fig. 2). Vaccinated patients had significantly less frequent fatigue ($p < 0.001$) and combination of several (≥ 3) multiorgan symptoms ($p < 0.001$).

Table 2 shows the clinical laboratory data. There were no differences between the vaccinated versus non-vaccinated patients. No conspicuous pathologic laboratory results were reported in any patients. The vast majority of circulating biomarkers remained in the normal range - including coagulation, hematologic, and cardiologic parameters. Marginally elevated laboratory values were followed-up with thorough clinical investigations to exclude organ diseases. In two patients, mildly elevated D-dimer was found to be due to mild obstructive pulmonary disease.

Table 2

Clinical lab data showing no difference between vaccinated and non-vaccinated patients.

Routine clinical lab data	All patients (*n* = 252)	Patients without vaccination (*n* = 97; 38%)	Patients already vaccinated before first clinical presentation (*n* = 155; 62%)
Hematology and general			
Hgb g/dL	14.0 ± 1.3	13.9 ± 1.3	14.1 ± 1.3
Platelet G/L	256 ± 55	258 ± 54	254 ± 56
Leukocyte G/L	6.6 ± 1.8	6.6 ± 1.9	6.5 ± 1.7
Creatinin mg/dL	0.78 ± 0.15	0.78 ± 0.17	0.77 ± 0.14
Albumin g/L	47.5 ± 2.8	47.5 ± 2.7	47.5 ± 2.9
SGOT U/L	24 ± 10	25 ± 12	24 ± 9
SGPT U/L	28 ± 23	30 ± 25	27 ± 22
Eisen ug/dL	92 ± 35.1	92.1 ± 38.8	91.9 ± 32.8
TSH u/U/mL	1.58 ± 0.93	1.57 ± 0.8	1.59 ± 1
Coagulation			
Prothrombin time %	98.7 ± 18.4	97.2 ± 17.3	99.6 ± 19.1
INR	1.1 ± 0.2	1.0 ± 0.1	1.1 ± 0.2
aPTT s	35.3 ± 4	35.7 ± 4.5	35.1 ± 3.7
Fibrinogen mg/dL	312 ± 66.8	307 ± 68	315 ± 66
D-dimer ug/mL	0 (0;0.39)	0 (0;0.35)	0.14 (0;0.41)
Elevated D-dimer	18/223 (8.1%)*	6/88 (6.8%)	12/135 (8.9%)
vWF antigen %	125 ± 53	129 ± 46	123 ± 57
ADAMTs13 activity %	107 ± 27	108 ± 28	106 ± 26
Cardiology			
Troponin T ng/L	0 (0;5)	0 (0;5)	0 (0;5.25)

Routine clinical lab data	All patients (*n* = 252)	Patients without vaccination (*n* = 97; 38%)	Patients already vaccinated before first clinical presentation (*n* = 155; 62%)
Elevated troponin T	6/240 (2.5%)**	3/93 (3.2%)	3/149 (2.0%)
Creatine kinase U/L	106 ± 66	103 ± 68	107 ± 65
NT-proBNP pg/mL	45.4 (28.5;85.1)	46.4 (26.3;80.4)	45.4 (29.9;92.8)
Elevated proBNP	15/243 (6.2%)***	5/92 (5.4%)	10/151 (6.6%)

Data are presented as mean ± SD, median (interquartile range), or number (frequency).

* Two patients were diagnosed with mild chronic obstructive pulmonary disease.

** Acute coronary syndrome was excluded in all patients.

*** Patients were thoroughly evaluated for cardiac and pulmonary disease.

Table 3 presents the circulating inflammatory biomarker levels. Acute infection was excluded in all patients. The mean and median values of the inflammatory biomarkers remained in the normal range. Compared to non-vaccinated patients, vaccinated patients showed a trend towards lower total IgG values, with significantly lower levels of IgG subfractions 2 and 4.

Table 3
Circulating inflammatory biomarker levels.

Inflammatory parameter	All patients (*n* = 252)	Patients without vaccination (*n* = 97; 38%)	Patients already vaccinated before first clinical presentation (*n* = 155; 62%)	p between w/wo vaccine
C-reactive protein mg/dL	0.09 (0.05;0.21)	0.09 (0.04;0.18)	0.11 (0.05;0.23)	
LDH U/L	166 (153;186)	166 (151;186)	167 (156;186)	
Ferritin ug/L	79.2 (40.1;151)	66.8 (33;151.8)	90.4 (46.4;152.7)	
Transferrin mg/dL	269 ± 42	264 ± 37	271 ± 45	
Transferrin saturation %	25.2 ± 11.4	25.4 ± 11.6	25.0 ± 11.3	
Histamin mg/dL	7.3 ± 2.6	7.0 ± 3.7	7.5 ± 3.5	
IL-6 pg/mL	1.57 (0;2.26)	1.57 (0;2.34)	1.56 (0;2.2)	
Procalcitonin ng/mL	0.03 (0;0.04)	0.03 (0;0.04)	0.03 (0;0.04)	
Total IgG mg/dL	1125 ± 283	1168 ± 355	1098 ± 224	0.066
Total IgA mg/dL	204 ± 84	212 ± 87	199 ± 82	
Total IgM mg/dL	107 ± 51	108 ± 63	107 ± 50	
Total IgE kIU/L	27.7 (11.7;78.4)	33.6 (10.9;86.8)	26 (12.9;74.1)	
IgG-1 subfraction mg/dL	700 ± 191	721 ± 241	688 ± 151	
IgG-2 subfraction mg/dL	347 ± 139	373 ± 165	331 ± 118	**0.026**
IgG-3 subfraction mg/dL	37 ± 21	38.2 ± 24.4	36.3 ± 17.8	
IgG-4 subfraction mg/dL	61 ± 56	72.2 ± 68.8	53.3 ± 44.4	**0.014**

Inflammatory parameter	All patients (*n* = 252)	Patients without vaccination (*n* = 97; 38%)	Patients already vaccinated before first clinical presentation (*n* = 155; 62%)	p between w/wo vaccine
Tryptase ug/L	4.8 ± 2.8	4.7 ± 2.7	4.8 ± 2.9	
Rheumafactor Latex IU/mL	0 (0;0)	0 (0;0)	0 (0;0)	
Alpha 1 antitrypsin mg/dL	136 ± 23	137 ± 21	135 ± 25	
Cardiolipin IgG U/mL	1.3 (1.1;1.7)	1.3 (1.1;1.7)	1.3 (1.1;1.7)	
Cardiolipin IgM U/mL	1.4 (1;2.25)	1.4 (0;2)	2.4 (1;2.3)	

Data are presented as mean ± SD or median (interquartile range).

Table 4 presents the qualitative and quantitative plasma virus titers in all patients, and in the subgroups. Among all patients, 15.1% presented cumulative IgM positivity or elevated virus-specific PCR level, as did 21.6% of non-vaccinated patients versus 11% of vaccinated patients ($p = 0.029$). Among all patients, 34.4% and 36.3%, respectively, presented with higher EBV and HSV nuclear antigen IgG titers, above the detection limit of the laboratory investigation, which might be interpreted as reactivations of EBV or HSV infections, as supported by the current literature [19]. Vaccination was associated with significantly lower cumulative IgM positivity of the investigated viruses, and with less CMV IgG, CMV IgM, and EBV IgM. This suggested an anti-SARS-CoV-2 vaccine-induced decrease in the viral–viral interaction-triggered antibody production. Interestingly, the parvovirus B19 IgG titer was increased in vaccinated individuals.

Table 4
Peripheral blood qualitative and quantitative IgG and IgM virus titers.

Routine virology lab data	All patients (*n* = 252)	Patients without vaccination (*n* = 97; 38%)	Patients already vaccinated before first clinical presentation (*n* = 155; 62%)	p between w/wo vaccine
Quantitative data				
CMV IgG mg/dL	62.3 (0;116)	87.3 (0;119.0)	11.4 (0;114)	**0.044**
CMV IgM mg/dL	0 (0;0)	0 (0;5.9)	0 (0;0)	**0.004**
EBV IgG mg/dL	310 (105;750)	402 (136;750)	267 (99;750)	
EBV IgM mg/dL	0 (0;0)	0 (0;8.53)	0 (0;0)	**0.025**
EBV EBNA IgG mg/dL	242 (66;516)	272 (70;529)	220 (65;514)	
HSV IgG mg/dL	22.2 (0.9;30.0)	22.8 (1.0;30)	22.1 (0.8;30)	
HSV IgM mg/dL	0 (0;0)	0 (0;5.1)	0 (0;0)	
VZV IgG mg/dL	1076 (661;1577)	1108 (661;1567)	1068 (652;1584)	
VZV IgM mg/dL	0.19 (0.14;0.28)	0.22 (0.16;0.3)	0.18 (0.14;0.27)	0.087
Parvo_B19 IgG mg/dL	22 (2.4;43.0)	16 (0;39)	27 (3.4;46)	**0.028**
Parvo_B19 IgM mg/dL	0.20 (0;0.45)	0.25 (0;0.47)	0 (0.05;0.41)	0.056
Qualitative data				
Cumulative virus IgM positivity (n = 252)	38 (15.1%)	21 (21.6%)	17 (11.0%)	**0.029**
CMV IgG positivity (n = 235)	144 (61.3%)	59 (71.1%)	85 (55.9%)	**0.025**
CMV IgM positivity (n = 235)	8 (3.4%)	4 (4.8%)	4 (2.6%)	
EBV IgG positivity (n = 244)	236 (96.7%)	91 (97.8%)	145 (96.0%)	
EBV IgG positivity above detection limit (n = 244)	84 (34.4%)	37 (39.8%)	47 (31.1%)	

Routine virology lab data	All patients (*n* = 252)	Patients without vaccination (*n* = 97; 38%)	Patients already vaccinated before first clinical presentation (*n* = 155; 62%)	p between w/wo vaccine
Quantitative data				
EBV IgM positivity (n = 244)	10 (4.1%)	7 (7.5%)	3 (2.0%)	**0.046**
EBV EBNA IgG positivity (n = 244)	213 (87.3%)	83 (89.2%)	130 (86.1%)	
EBV EBNA IgG above detection limit (n = 244)	52 (21.3%)	20 (21.5%)	32 (21.2%)	
HSV IgG positivity (n = 237)	211 (89.0%)	79 (92.9%)	132 (86.8%)	
HSV IgG positivity above detection limit (n = 237)	86 (36.3%)	34 (41.0%)	52 (34.2%)	
HSV IgM positivity (n = 235)	8 (3.4%)	3 (3.7%)	5 (3.3%)	
VZV IgG positivity (n = 235)	235 (100%)	82 (100%)	153 (100%)	
VZV IgM positivity (n = 235)	2 (0.8%)	0 (0%)	2 (1.3%)	
Parvo_B19 IgG positivity (n = 247)	195 (78.9%)	71 (74.5%)	124 (81.6%)	
Parvo_B19 IgM positivity (n = 247)	15 (6.1%)	7 (7.4%)	8 (5.3%)	

Data are presented as mean ± SD, median (interquartile ranges), or number (frequency).

In contrast with the vaccinated population, non-vaccinated patients exhibited a temporary increase of cumulative IgM virus positivity (Fig. 3).

We observed a significant logarithmic correlation between the time to infection and quantitative EBV IgG titer, showing an increase of EBV IgG titer over time (Fig. 4), which was only marginally influenced by vaccination.

In contrast, the quantitative titer of parvovirus B19 IgM exhibited a linear decrease (Fig. 5).

The other lab values showed no time-dependent changes, including hematologic, coagulation, and inflammatory biomarkers, routine lab measurements (kidney, liver, cardiac, etc.), and viral titers at the first clinical presentation.

Virus IgG and IgM titers did not differ between the patient groups with dominant neuropsychiatric, pulmonary, or cardiologic symptoms, except in two cases. First, 47.4% of patients in the pulmonary group had an EBV VCA IgG titer over the detection limit, compared with 32.2% in the neuropsychiatric group and 18.5% in the cardiologic group ($p = 0.013$). Second, 90.9% of patients in the cardiologic group had parvovirus B19 IgG positivity, compared to 74.2% in the neuropsychiatric group and 84.2% in the pulmonary group ($p = 0.022$).

First substudy: Protective role of anti-SARS-CoV-2 vaccination in a subgroup of patients who received the vaccine before the COVID-19 infection

Supplementary Tables S1–S4 present the clinical and laboratory data of the subgroup of patients who received the vaccine prior to COVID-19 infection ("protected group"), compared with the patients who had infection before vaccination. Patients in the "protected group" exhibited significantly lower NT-proBNP and cardiolipin IgM. Immunoprotection before SARS-CoV-2 infection was associated with significantly lower EBV IgG, lower incidence of EBV IgG positivity, and lower frequency of having high EBV VCA IgG over the detection limit (Suppl. Table S4).

Second substudy: Patients with repeated evaluation of clinical presentation and blood sampling

A total of 131 patients had a second assessment of clinical presentation with blood sampling, with an interval of 106 ± 77 days between the baseline and follow-up blood sampling times. Clinical laboratory values did not differ between the first and second blood sampling. Among these patients, from the first to the second blood sampling time, we observed significant decreases in the cumulative virus IgM positivity (from 19.8–11.5%, $p = 0.044$), and in the parvovirus B19 IgM titer (from 0.45 ± 1.7 mg/dL to 0.21 ± 0.32 mg/dL, $p = 0.019$).

Among these 131 patients, 72 were already vaccinated at the first clinical presentation, and an additional 34 patients received their first vaccination between the first and second blood sampling. Considering, that vaccination influenced the viral IgG and IgM titers (see main study above), the vaccinations received before and after the first blood sampling might cause a bias in the interpretation of these results. Only 25 patients remained unvaccinated at the time of their second blood sampling. Among these 25 patients, the only significant difference between the first and second blood sampling was an increase in parvovirus B19 IgG positivity from 68–100% ($p = 0.004$).

Third substudy: Comparison of plasma viral antibody titers between long-COVID patients and healthy non-vaccinated non-infected controls

For the first 105 consecutive patients with long-COVID syndrome (age 46 ± 15 years, 36.2% male), clinical data and blood samples were collected between March 15th 2021 and September 30th 2021. Blood samples of age- and sex -matched (46 ± 12 years, 36.2% male) healthy individuals, collected during the period between June 18th 2020 and Nov. 11th 2020 (EC: 1387/2020; ClinicalTrials.gov Identifier:

NCT04407429) [30] were retrieved from the Biobank facility of the Medical University of Vienna (processing and storage in accordance with the standard operating procedures and an ISO 9001:2015) [31]. Information on sex and age were obtained through the hospital electronic database. These individuals were not yet vaccinated, and had no spike protein or nucleocapsid antibodies, indicating no previous COVID-19 infection. For all long-COVID patients, the time between SARS-CoV-2 infection and their first clinical visit was 219 ± 98 days (7 ± 3 months). Anti-spike protein antibody was zero in healthy controls, and 1162.6 ± 1150.7 BAU/mL among all long-COVID patients. Table 5 shows the qualitative results. Figure 6 presents the box plots of the quantitative IgG and IgM virus titers, revealing significantly higher EBV VCA IgG titers in long-COVID patients compared to in healthy controls ($p = 0.033$). Interestingly, the long-COVID patients had a significantly lower parvovirus B19 IgG titer, but a significantly higher IgM titer ($p < 0.001$) (Fig. 6 and Table 5).

Table 5
Qualitative IgG and IgM titers of the investigated viruses among patients with long-COVID syndrome and healthy (non-infected, non-vaccinated) controls.

	Healthy (*n* = 105)	**Long COVID (*n* = 105)**	***P* value**
male	38 (36.2%)	38 (36.2%)	
Cumulative virus IgM positivity	7 (6.7%)	19 (18.1%)	**0.02**
CMV IgG positivity	60 (57.1%)	59/103 (57.3%)	
CMV IgM positivity	1 (1%)	4/103 (3.9%)	
EBV IgG positivity	100 (95,2%)	103 (98.1)	
EBV VCA IgG positivity above detection limit	25 (23.8%)	42 (40.0%)	**0.018**
EBV IgM positivity	2 (1.9%)	7 (6.7%)	
EBV EBNA IgG positivity	91 (86.7%)	100 (95.2%)	
EBV EBNA IgG above detection limit	21 (20%)	18 (17.1%)	
HSV IgG positivity	87 (82.9%)	82/104 (78.8%)	
HSV IgG positivity above detection limit	44 (41.9%)	40/104 (38.5%)	
HSV IgM positivity	2 (1.9%)	6/104 (5.8%)	
VZV IgG positivity	105 (100%)	98/101 (97%)	
VZV IgM positivity	2 (1.9%)	1/101 (1%)	
Parvo_B19 IgG positivity	91 (86.7%)	86 (81.9%	
Parvo_B19 IgG positivity above detection limit	24 (22.9%)	14 (13.3%)	
Parvo_B19 IgM positivity	1 (1%)	6 (5.7%)	

Discussion

Main findings

In this study of patients with long-COVID syndrome, one of our main findings is that SARS-CoV-2 infection apparently activated certain types of DNA viruses (EBV, HSV, CVM, and parvovirus-B19), as demonstrated by the significantly higher incidence of cumulative IgM positivity, and elevated EBV VCA IgG and parvovirus-B19 IgM titers, in long-COVID patients compared to healthy controls. Overall, 34.4% and 36.3% of patients, respectively, presented with higher EBV and HSV nuclear antigen IgG titers, over the detection limit of the commercially available laboratory tests. The time to infection exhibited a significant logarithmic correlation with quantitative EBV IgG titer, with the EBV IgG titer increasing over time after SARS-CoV-2 infection. In contrast, the parvovirus-B19 IgM quantitative titer decreased linearly with increasing time after COVID-19.

Another main finding of our study is that anti-SARS-CoV-2 vaccination played a protective role against DNA virus activations (EBV, HSV, CVM, and parvovirus-B19), as proven at the patient level. In detail, compared to patients who were not vaccinated, the patients who were vaccinated against SARS-CoV-2 during their long-COVID phase exhibited 1) significantly less frequent fatigue and combination of several multiorgan symptoms; 2) significantly lower plasma levels of IgG subfractions 2 and 4; 3) significantly less frequent cumulative IgM positivity or positive virus-specific PCR titer; and 4) significantly lower quantitative CMV IgG, CMV IgM, and EBV IgM titers. Moreover, among vaccinated individuals, compared to patients vaccinated during their long-COVID phase, patients who were already immunoprotected against SARS-CoV-2 before their first COVID-19 infection exhibited significantly lower EBV VCA IgG titer, lower NT-proBNP plasma level, and lower cardiolipin IgM titer.

Comparison of our data with literature data

Several previous studies have reported the co-detection of different viruses (mainly respiratory viruses) in severely ill hospitalized patients infected with SARS-CoV-2 [32]. However, approximately 92% of COVID-19 patients with mild or moderate symptoms remained quarantined at home, without any medical records, and no information available about possible co-infections with viruses or other pathogens that might affect the long-term outcomes and development of long-COVID syndrome [33]. The long-COVID phase lasts several months, or even years, and can include multiorgan symptoms of variable degrees, which is not typical for an acute infection. Therefore, routine clinical investigations for actual pathogen infection are not clinically justified. However, some long-COVID symptoms resemble subclinical post-viral symptoms, such as chronic fatigue syndrome, low-grade fever, rapid exhaustion, and post-exertional malaise. Several publications have suggested that these long-lasting symptoms may be caused by the sequential and prolonged subclinical activation of viruses that are normally co-localized in the nasopharyngeal space [16, 17, 18, 19, 20, 21].

Peluso et al reported the effect of pre-existing or chronic viral load, and reactivation of EBV and CMV, on neurocognitive and fatigue symptoms at a median of 4 months after COVID-19 infection, and suggested

EBV IgG and EBNA as a potential biomarker of EBV reactivation (Peluso [21]). Moreover, EBV infection and reactivation may induce autoimmune processes that could further explain chronic subclinical inflammation and related symptoms in long-COVID patients [21]. Similar to the findings of Peluso et al, we also observed that a high proportion of patients had EBV IgG and EBNA titers above the detection limit.

Notably, in our present study, we excluded patients with significant co-morbidities, such as HIV infection, and patients who were hospitalized due to severe COVID-19 infection. Moreover, we matched control patients to our long-COVID patients, and investigated the effect of the COVID-19 vaccination on several viral titers. While the beneficial effects of vaccination on long-COVID syndrome have already been extensively investigated, the presently observed protective effect of vaccination against SARS-CoV-2-associated reactivation of other viruses has not previously been reported. Additionally, our patient cohort had a longer follow-up (median of 8 months post-infection), and many patients had an elevated and positive IgM titer several months after their initial SARS-CoV-2 infection. This raises the question of whether these patients' symptoms are consequences of the prolonged viral–viral interaction, or if the "co-infection" is independent from the initial SARS-CoV-2 infection and simply a new viral infection in patients with altered immune responses after COVID-19 illness.

RNA–DNA viral interaction after SARS-CoV-2 infection and the role of vaccination

In our study, vaccination against SARS-CoV-2 (an RNA virus) was associated with decreased DNA viral antibody titers, suggesting that anti-SARS-CoV-2 vaccination may interrupt viral–viral communication. Although the exact mechanism is only speculative, there are several proposals for how an RNA virus could activate other RNA or DNA viruses [34], and how an anti-RNA virus molecule may de-activate this viral interference. RNA and DNA viruses have different cellular receptors that affect diverse signaling mechanisms within the innate immune response. However, there is cross-talk between the mechanisms of detection of nucleic acids originating from RNA and DNA viruses, and downstream regulators. This might at least partly explain the amplification of their interactions [35, 34] -for example, suppressing the host antiviral reaction, and thereby facilitating the invasion of co-occurring viruses - which, in turn, can lead to parallel de-activation of several co-localized viruses. However, contradicting the assumption that SARS-CoV-2 induces reactivation of other viral pathogens, Burstein et al showed in a large-scale study that virus pairs do not act synergically, and rather mitigate their infective capacity [36]. For example, acute SARS-CoV-2 infection has reportedly attenuated the rhinovirus (a rapidly replicating virus) viral load, suggesting the competitive consumption of "cellular nutritional resources" and interference between viral pathogens [37, 34]. There remains a need for systemic investigations to elucidate the exact mechanisms of viral interference, and its importance in terms of long-term morbidity and outcomes of patients with long-COVID syndromes.

In conclusion, the results of our present clinical investigation provide the first demonstration of parallel or sequential activation of DNA viruses after SARS-CoV-2 clinical infection (viral cross-talk or interference).

We further show the interruption of this viral cross-talk by anti-SARS-CoV2 vaccination in patients with long-COVID syndrome.

Methods

Study design and patients

The POSTCOV cohort study is an on-going multicenter prospective registry (EC: 1008/2021). For data control, the study was extended with a case-control study (EC: 1387/2020). The presentation of the methods and results is conforms with the STROBE guidelines [38].

Inclusion and exclusion criteria

Inclusion criteria for long-COVID patients were as follows: 1) previous COVID-19 infection confirmed by quantitative real-time polymerase chain reaction (PCR); 2) previous mild or moderate COVID-19 illness not requiring hospitalization; 3) absence of previous or present inflammatory disease, malignancies, or chronic organ disorders (e.g., renal insufficiency, chronic heart or lung disease, or rheumatic diseases); and 4) at least three different organ-related symptoms fulfilling the criteria of long-COVID syndrome. Exclusion criteria were as follows: 1) clinically proven active infection combined with elevated inflammatory markers, such as C-reactive protein (CRP), leukocytes, and fibrinogen; 2) no verified past SARS-CoV2 infection, or missing PCR test; 3) clinical acute infection of any kind independent from laboratory values; and 4) any kind of known or clinically proven active chronic diseases or malignancies, under previous or current disease-specific treatments.

Clinical data

Clinical and laboratory data were collected, including via blood sampling, at the time of the first clinical presentation between April 2021 and May 2022. The following clinical data were recorded: age, gender, time of COVID positivity, time and type of vaccine, time between COVID positivity and blood sampling, long-COVID disease type (neuropsychological, pulmonary, or cardiovascular) (cit EHJ???), atherosclerotic risk factors (diabetes mellitus, hypertension, hyperlipidemia, and smoking), systolic and diastolic blood pressure, heart rate, and ECG abnormalities.

Laboratory data

All patients underwent venous blood sampling at the first clinical presentation, or at the control clinical visit. Clinical laboratory data; inflammatory, hematologic, and coagulation parameters; and cardiac biomarkers were assessed. Clinical virology parameters—such as virus-specific immunoglobulin (Ig) IgG or IgM, or PCR of CMV, EBV VCA, HS, VZ, and parvovirus-B19, and EBV EBNA—were measured, and the results were reported qualitatively and quantitatively. All laboratory investigations were performed at the Department of Laboratory Medicine, Medical University of Vienna, Wien, Austria. Detailed laboratory methods are described on the institution homepage (https://www.akhwien.at/default.aspx?pid=3985).

Substudies

Three substudies were performed. First, we evaluated the protective effect of the vaccine when a vaccinated person was infected with COVID-19 (“protected”), compared with patients who received the vaccine after COVID-19 infection. Second, we analyzed patients who underwent repeated evaluations of clinical presentation and blood sampling (n = 131). Third, we compared COVID-19 patients with healthy unvaccinated age- and gender-matched control individuals (n = 105 of each group). This sub-analysis included data from the first consecutive long-COVID patients.

Statistical analyses

Continuous parameters were reported as mean ± standard, and nominal data as frequency with percentage (%). Several patients had quantitative IgG antibody titers over the detection limit, and the maximal detection limit value was calculated for quantitative analyses of these patients (https://bvcentre.ca/files/research_reports/08-03GuidanceDocument.pdf). Quantitative values below the detection limit (reported as lower than the detection limit) were calculated as zero. Anti-spike protein antibody was measured at the time of the first clinical presentation. Differences between the groups were calculated using the two-sided non-parametric Mann-Whitney test for data with a non-normal distribution, or two-sided Student’s t-test for data with a normal distribution, and the chi-squared test for nominal variables. For statistical analyses, SPSS Version 28.0.1.0 (142) was used. A P value of < 0.05 was considered to indicate statistical significance.

Declarations

COMPETING INTERESTS

The current study was sponsored by the Austrian Science Fund KLI 1064-B and Medical-Scientific Fund of the Mayor of Vienna City 21176 to Gy.M., and Austrian Science Fund KLI 876-B to T.A.Z.

M.G. received honoraria for long COVID lectures form the following companies: General Reinsurance AG, Genericon Pharma, Österreichische Apotheker-Verlagsgesellschaft m.b.H.

REPORTING SUMMARY

Information on research design is available in the Nature Research Reporting Summary linked to this article.

DATA AVAILABILITY

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

AUTHOR CONTRIBUTIONS

M.G., J.B-K., M.R., C.H., E.P-S., E.H. and T.A.Z. conceptualized the trial.

M.G., A.S., V.S., E.S., J.B-K., M.R., C.N., E.H. and T.A.Z. coordinated the study.

D.L., J.M-T., K.Z., P.E., A.S., V.S., K.S., M.R., C.N., P.M., H.L., M.B., R.S., C.L., D.B., E.H. and T.A.Z. conducted the trial.

D.L., J.M-T., K.Z., P.E., A.S., V.S., K.S., M.R., C.N., P.M., H.L., M.B., R.S., C.L., D.B. and E.H. processed the clinical specimens and conducted the laboratory analyses.

D.L., J.M-T., K.Z. A.S., V.S., E.S., J.B-K., M.R., C.N. and C.H. obtained regulatory approval.

M.G., D.L., P.E., A.S., V.S., K.S., J.B-K., M.R., C.N., P.M., H.L., M.B., R.S., E.P-S., C.L., D.B., E.H. and T.A.Z. analyzed and interpreted the data.

M.G. and E.H. drafted the manuscript.

M.G., E.H. and T.A.Z. finalized the manuscript.

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The current study was sponsored by the Austrian Science Fund KLI 1064-B and Medical-Scientific Fund of the Mayor of Vienna City 21176 to Gy.M., and Austrian Science Fund KLI 876-B to T.A.Z.

References

1. Jacobs, J. J. L. Persistent SARS-2 infections contribute to long COVID-19. *Med Hypotheses* **149**, 110538 (2021). https://doi.org:10.1016/j.mehy.2021.110538
2. Hatayama, Y., Hashimoto, Y. & Motokura, T. Frequent co-reactivation of Epstein-Barr virus in patients with cytomegalovirus viremia under immunosuppressive therapy and/or chemotherapy. *J Int Med Res* **48**, 300060520972880 (2020). https://doi.org:10.1177/0300060520972880
3. Libert, N. *et al.* Epstein-Barr virus reactivation in critically ill immunocompetent patients. *Biomed J* **38**, 70-76 (2015). https://doi.org:10.4103/2319-4170.132905
4. Guo, L. *et al.* Recent Advances in HBV Reactivation Research. *Biomed Res Int* **2018**, 2931402 (2018). https://doi.org:10.1155/2018/2931402
5. Lehner, G. F. *et al.* Correlation of interleukin-6 with Epstein-Barr virus levels in COVID-19. *Crit Care* **24**, 657 (2020). https://doi.org:10.1186/s13054-020-03384-6
6. Chen, T., Song, J., Liu, H., Zheng, H. & Chen, C. Positive Epstein-Barr virus detection in coronavirus disease 2019 (COVID-19) patients. *Sci Rep* **11**, 10902 (2021). https://doi.org:10.1038/s41598-021-90351-y
7. Perez-Granda, M. J. *et al.* Cytomegalovirus reactivation in patients diagnosed with severe COVID-19: A point prevalence study in a general hospital. *Rev Esp Quimioter* **36**, 45-51 (2023). https://doi.org:10.37201/req/068.2022

8. Siddiqui, S. S. *et al.* Cytomegalovirus Coinfection in Critically Ill Patients with Novel Coronavirus-2019 Disease: Pathogens or Spectators? *Indian J Crit Care Med* **26**, 376-380 (2022). https://doi.org:10.5005/jp-journals-10071-24130
9. Fuest, K. E. *et al.* Risk factors for Herpes simplex virus (HSV) and Cytomegalovirus (CMV) infections in critically-ill COVID-19 patients. *Multidiscip Respir Med* **17**, 815 (2022). https://doi.org:10.4081/mrm.2022.815
10. Simonnet, A. *et al.* High incidence of Epstein-Barr virus, cytomegalovirus, and human-herpes virus-6 reactivations in critically ill patients with COVID-19. *Infect Dis Now* **51**, 296-299 (2021). https://doi.org:10.1016/j.idnow.2021.01.005
11. Drago, F., Ciccarese, G., Rebora, A. & Parodi, A. Human herpesvirus-6, -7, and Epstein-Barr virus reactivation in pityriasis rosea during COVID-19. *J Med Virol* **93**, 1850-1851 (2021). https://doi.org:10.1002/jmv.26549
12. Cabrera Muras, A. *et al.* Bilateral facial nerve palsy associated with COVID-19 and Epstein-Barr virus co-infection. *Eur J Neurol* **28**, 358-360 (2021). https://doi.org:10.1111/ene.14561
13. Paolucci, S. *et al.* EBV DNA increase in COVID-19 patients with impaired lymphocyte subpopulation count. *Int J Infect Dis* **104**, 315-319 (2021). https://doi.org:10.1016/j.ijid.2020.12.051
14. Galestanian, A., Suthar, K. H. & Karnath, B. Immune Thrombocytopenic Purpura in a Patient With SARS-CoV-2 and Epstein-Barr Virus. *Cureus* **13**, e13615 (2021). https://doi.org:10.7759/cureus.13615
15. Roncati, L., Lusenti, B., Nasillo, V. & Manenti, A. Fatal SARS-CoV-2 coinfection in course of EBV-associated lymphoproliferative disease. *Ann Hematol* **99**, 1945-1946 (2020). https://doi.org:10.1007/s00277-020-04098-z
16. Gold, J. E., Okyay, R. A., Licht, W. E. & Hurley, D. J. Investigation of Long COVID Prevalence and Its Relationship to Epstein-Barr Virus Reactivation. *Pathogens* **10** (2021). https://doi.org:10.3390/pathogens10060763
17. Su, Y. *et al.* Multiple early factors anticipate post-acute COVID-19 sequelae. *Cell* **185**, 881-895 e820 (2022). https://doi.org:10.1016/j.cell.2022.01.014
18. Rohrhofer, J. *et al.* Association between Epstein-Barr-Virus reactivation and development of Long-COVID fatigue. *Allergy* **78**, 297-299 (2023). https://doi.org:10.1111/all.15471
19. Klein, J. *et al.* Distinguishing features of Long COVID identified through immune profiling. *medRxiv* (2022). https://doi.org:10.1101/2022.08.09.22278592
20. Nunn, A. V. W., Guy, G. W., Botchway, S. W. & Bell, J. D. SARS-CoV-2 and EBV; the cost of a second mitochondrial "whammy"? *Immun Ageing* **18**, 40 (2021). https://doi.org:10.1186/s12979-021-00252-x
21. Peluso, M. J. *et al.* Chronic viral coinfections differentially affect the likelihood of developing long COVID. *J Clin Invest* **133** (2023). https://doi.org:10.1172/JCI163669
22. Fernandez-de-Las-Penas, C. *et al.* Differences in Long-COVID Symptoms between Vaccinated and Non-Vaccinated (BNT162b2 Vaccine) Hospitalized COVID-19 Survivors Infected with the Delta Variant. *Vaccines (Basel)* **10** (2022). https://doi.org:10.3390/vaccines10091481

23. Kuodi, P. *et al.* Association between BNT162b2 vaccination and reported incidence of post-COVID-19 symptoms: cross-sectional study 2020-21, Israel. *NPJ Vaccines* **7**, 101 (2022). https://doi.org:10.1038/s41541-022-00526-5
24. Verma, D., Church, T. M. & Swaminathan, S. Epstein-Barr Virus Lytic Replication Induces ACE2 Expression and Enhances SARS-CoV-2 Pseudotyped Virus Entry in Epithelial Cells. *J Virol* **95**, e0019221 (2021). https://doi.org:10.1128/JVI.00192-21
25. Meskill, S. D. & O'Bryant, S. C. Respiratory Virus Co-infection in Acute Respiratory Infections in Children. *Curr Infect Dis Rep* **22**, 3 (2020). https://doi.org:10.1007/s11908-020-0711-8
26. Greenhalgh, T., Knight, M., A'Court, C., Buxton, M. & Husain, L. Management of post-acute covid-19 in primary care. *BMJ* **370**, m3026 (2020). https://doi.org:10.1136/bmj.m3026
27. Herrera, J. E. *et al.* Response to letter to the editor regarding "Multi-Disciplinary collaborative consensus guidance statement on the assessment and treatment of fatigue in patients with Post-Acute sequelae of SARS-CoV-2 infection (PASC)". *PM R* **13**, 1439-1440 (2021). https://doi.org:10.1002/pmrj.12719
28. Shah, Y. S. *et al.* An Algorithm for Ramp Up of Ophthalmic Elective Surgeries Post-COVID-19. *Ophthalmic Epidemiol* **28**, 90-92 (2021). https://doi.org:10.1080/09286586.2020.1832236
29. Gyongyosi, M. *et al.* Long COVID and the cardiovascular system - elucidating causes and cellular mechanisms in order to develop targeted diagnostic and therapeutic strategies: A joint Scientific Statement of the ESC Working Groups on Cellular Biology of the Heart and Myocardial & Pericardial Diseases. *Cardiovasc Res* (2022). https://doi.org:10.1093/cvr/cvac115
30. Riesenhuber, M. *et al.* Comparison of the prevalence of SARS-CoV-2 nucleoprotein antibodies in healthcare workers and an unselected adult and paediatric all-comer patient population: insights from a longitudinal study of healthcare workers and concurrent serial cross-sectional studies of patients at an academic medical centre in Austria. *BMJ Open* **13**, e063760 (2023). https://doi.org:10.1136/bmjopen-2022-063760
31. Haslacher, H. *et al.* Usage Data and Scientific Impact of the Prospectively Established Fluid Bioresources at the Hospital-Based MedUni Wien Biobank. *Biopreserv Biobank* **16**, 477-482 (2018). https://doi.org:10.1089/bio.2018.0032
32. Esper, F. P., Spahlinger, T. & Zhou, L. Rate and influence of respiratory virus co-infection on pandemic (H1N1) influenza disease. *J Infect* **63**, 260-266 (2011). https://doi.org:10.1016/j.jinf.2011.04.004
33. Xie, Y., Xu, E., Bowe, B. & Al-Aly, Z. Long-term cardiovascular outcomes of COVID-19. *Nat Med* **28**, 583-590 (2022). https://doi.org:10.1038/s41591-022-01689-3
34. Cai, C., Tang, Y. D., Xu, G. & Zheng, C. The crosstalk between viral RNA- and DNA-sensing mechanisms. *Cell Mol Life Sci* **78**, 7427-7434 (2021). https://doi.org:10.1007/s00018-021-04001-7
35. Latif, M. B., Raja, R., Kessler, P. M. & Sen, G. C. Relative Contributions of the cGAS-STING and TLR3 Signaling Pathways to Attenuation of Herpes Simplex Virus 1 Replication. *J Virol* **94** (2020). https://doi.org:10.1128/JVI.01717-19

36. Burstein, R. *et al.* Interactions among 17 respiratory pathogens: a cross-sectional study using clinical and community surveillance data. *medRxiv* (2022). https://doi.org:10.1101/2022.02.04.22270474
37. Hebert-Dufresne, L. & Althouse, B. M. Complex dynamics of synergistic coinfections on realistically clustered networks. *Proc Natl Acad Sci U S A* **112**, 10551-10556 (2015). https://doi.org:10.1073/pnas.1507820112
38. Zavada, J., Dixon, W. G., Askling, J., Registers, E. S. g. o. L. O. & Drug, S. Launch of a checklist for reporting longitudinal observational drug studies in rheumatology: a EULAR extension of STROBE guidelines based on experience from biologics registries. *Ann Rheum Dis* **73**, 628 (2014). https://doi.org:10.1136/annrheumdis-2013-204102

Figures

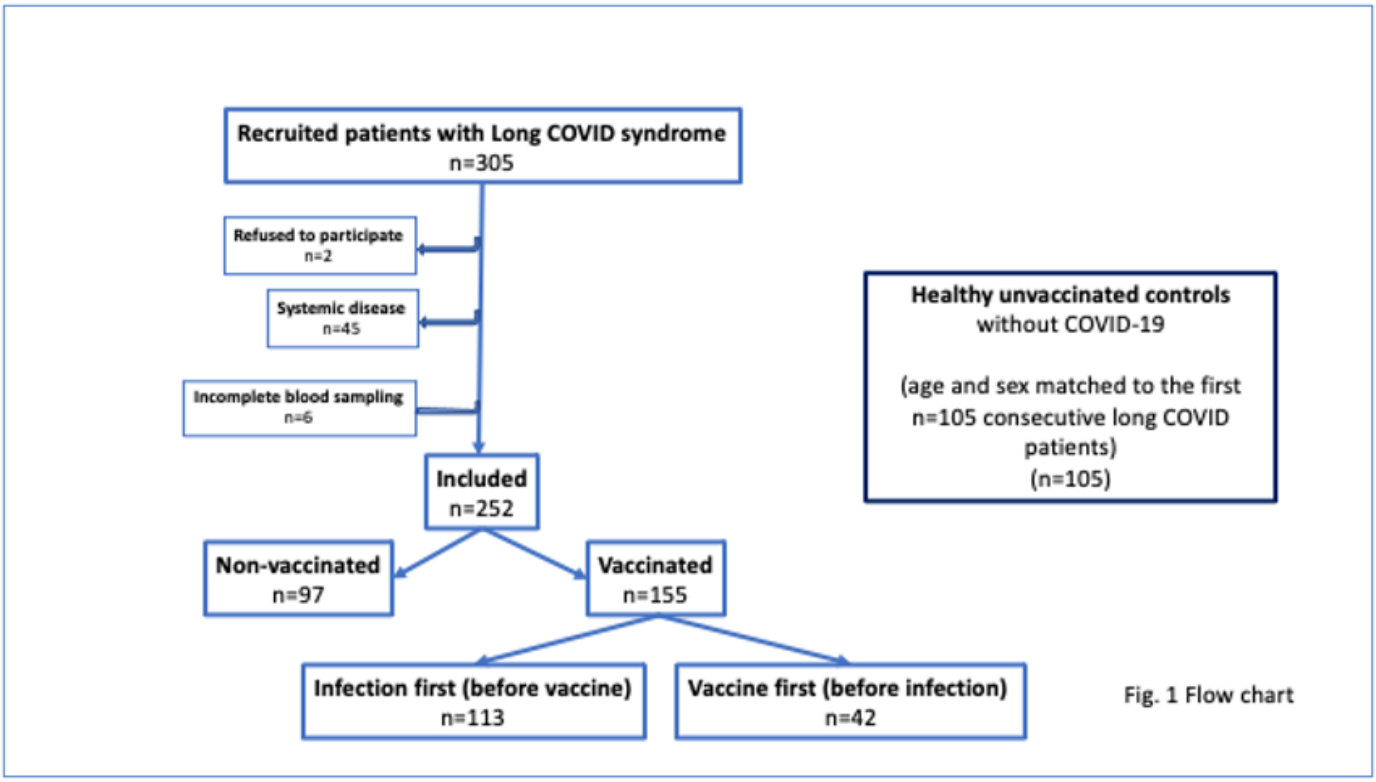

Figure 1

Flow chart of the study.

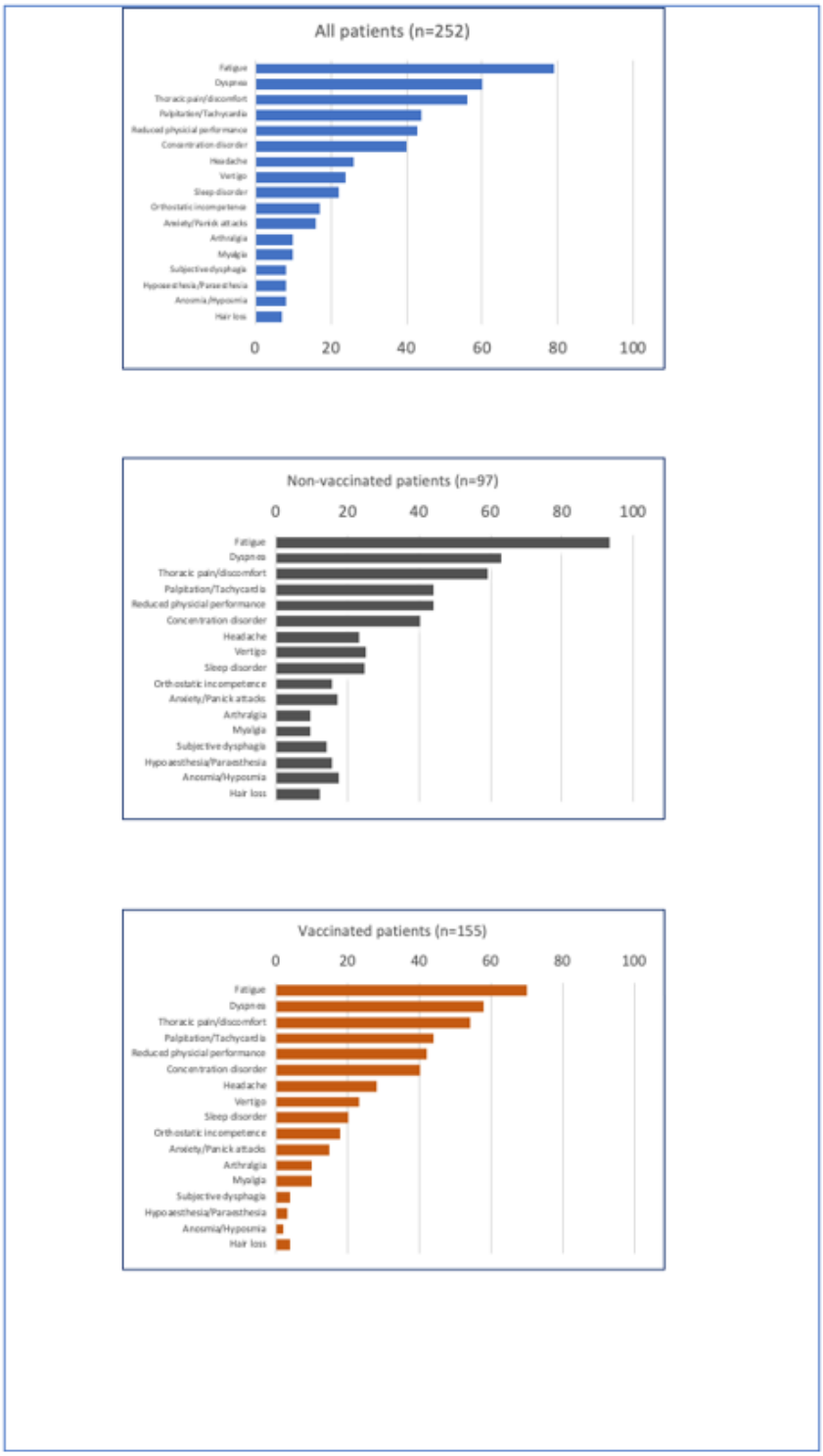

Figure 2

Clinical symptoms at the first clinical presentation.

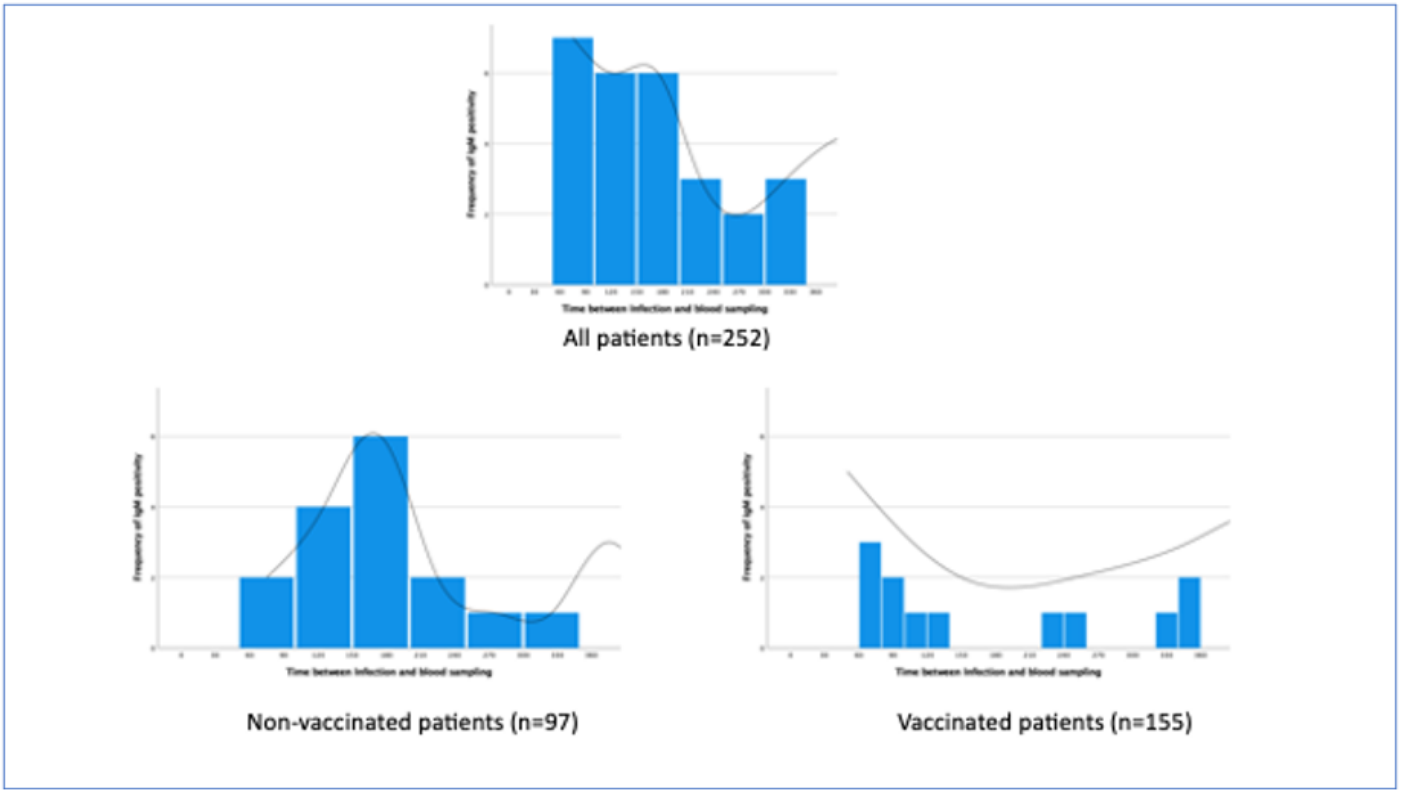

Figure 3

Cumulative IgM positivity, including herpes simplex virus (HSV), varicella-zoster virus (VZV), cytomegalovirus (CMV), Epstein-Barr virus (EBV), and parvovirus B19.

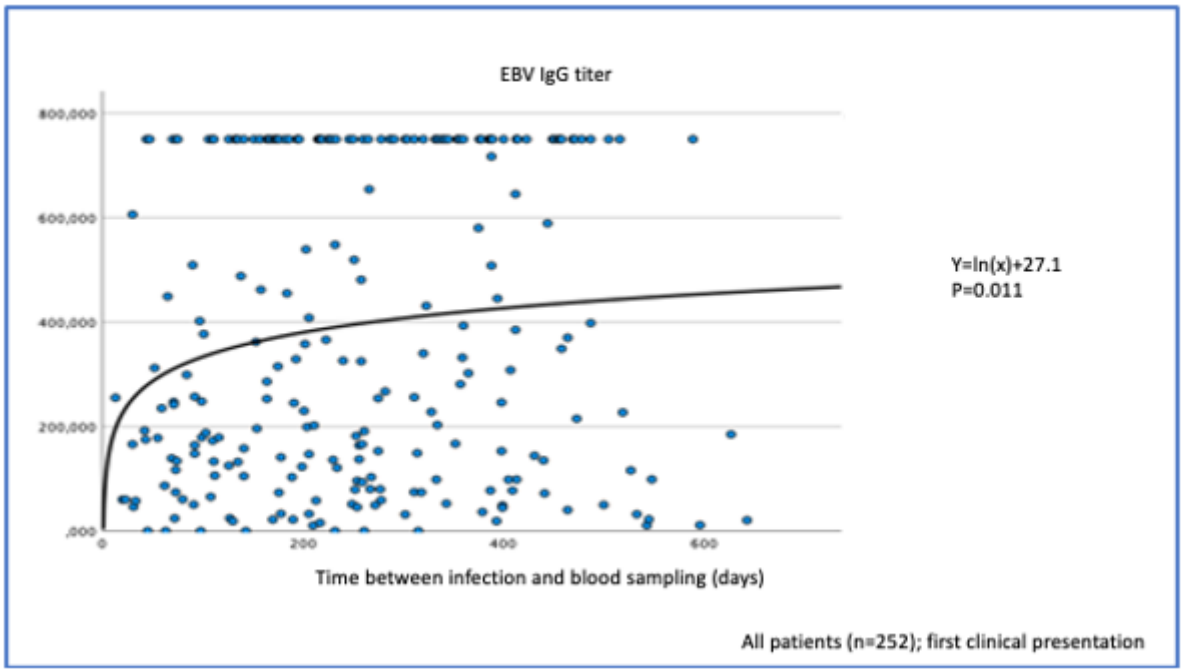

Figure 4

Quantitative Epstein-Barr virus (EBV) IgG titer (detection limit: 750 mg/dL) (*n*=252).

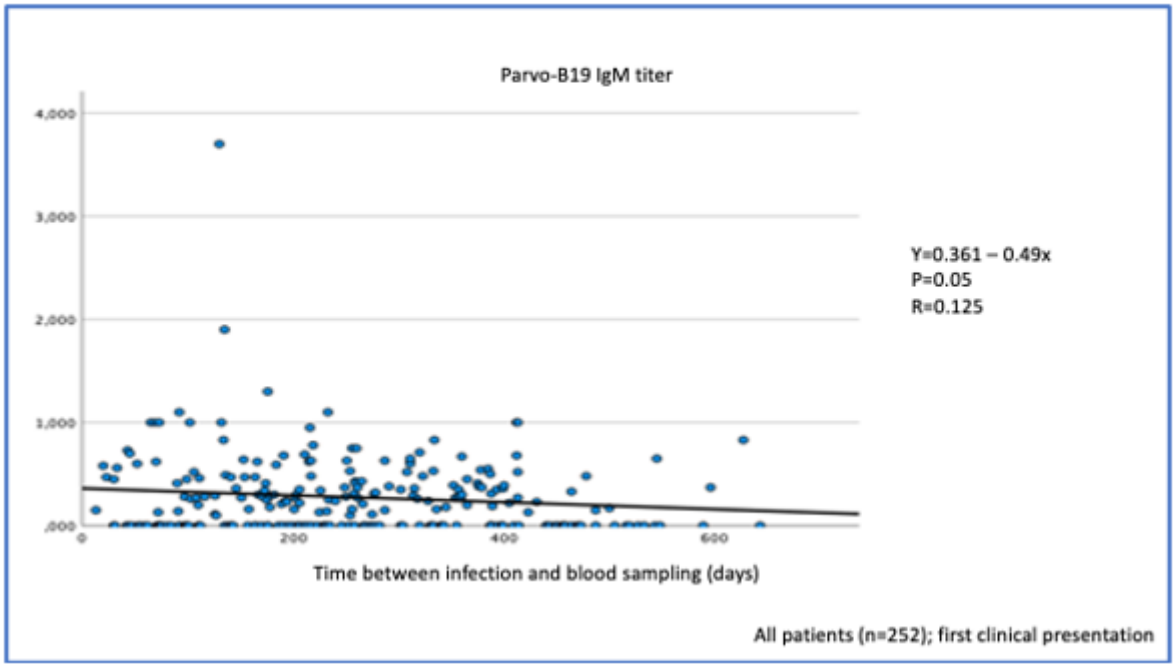

Figure 5

Quantitative parvovirus B19 IgM titer (*n*=247).

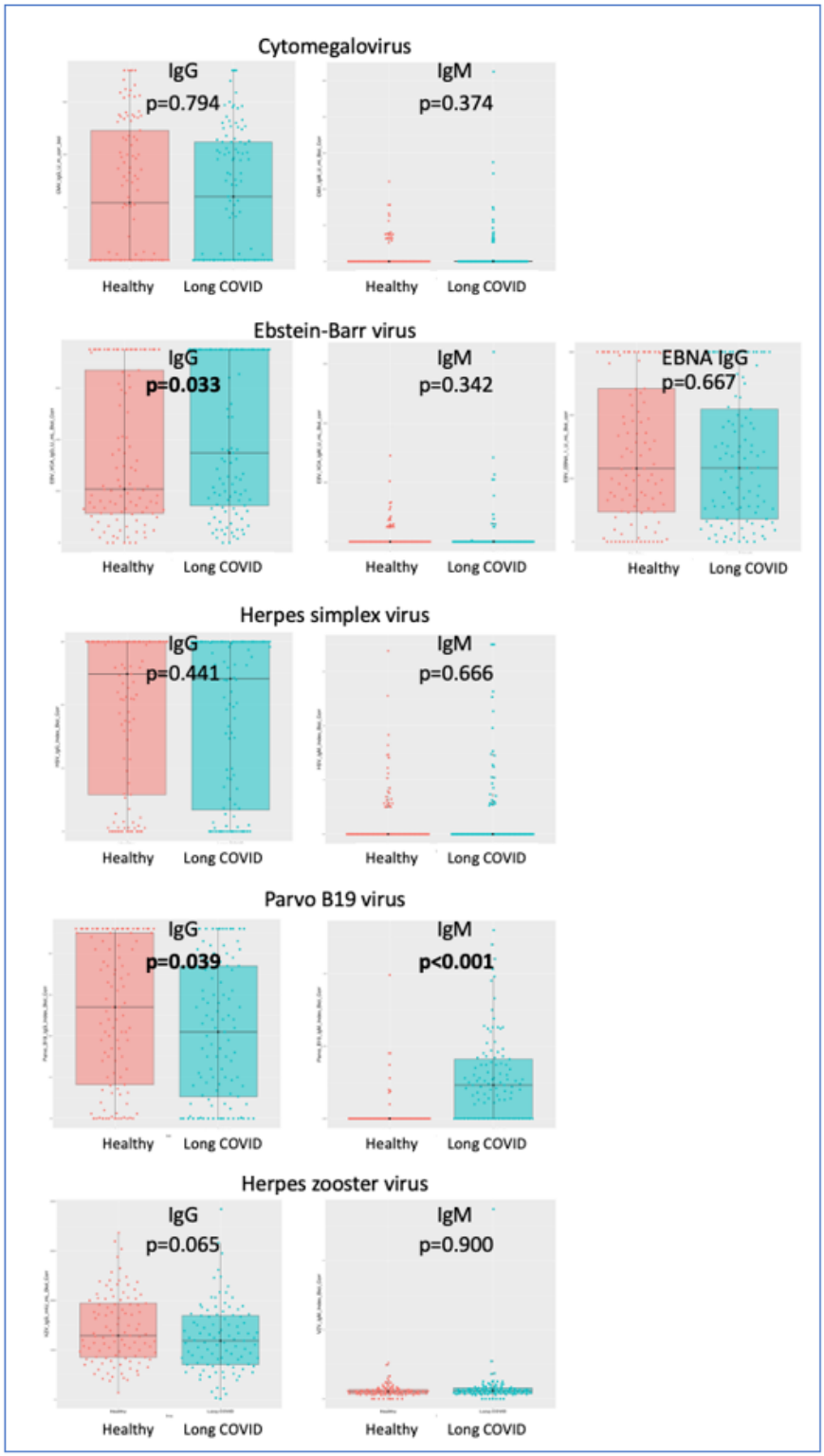

Figure 6

IgG and IgM virus titers (mg/dL) among patients with long COVID (*n* = 105) and age- and sex-matched healthy controls (*n* = 105).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- GyongyosiNPJVACCINES02759Suppl.file.docx