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Short communication

High incidence of Epstein–Barr virus, cytomegalovirus, and human-herpes virus-6 reactivations in critically ill patients with COVID-19

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ABSTRACT

Background: Systemic reactivation of herpesviruses may occur in intensive care unit (ICU) patients and is associated with morbidity and mortality. Data on severe Coronavirus disease-19 (COVID-19) and concomitant reactivation of herpesviruses are lacking.
Methods: We selected patients admitted to ICU for confirmed COVID-19 who underwent systematic testing for Epstein–Barr virus (EBV), cytomegalovirus (CMV) and human-herpes virus-6 (HHV-6) DNAemia while in the ICU. We retrospectively analysed frequency, timing, duration and co-occurrence of viral DNAemia.
Results: Thirty-four patients were included. Viremia with EBV, CMV, and HHV-6 was detected in 28 (82%), 5 (15%), and 7 (22%) patients, respectively. EBV reactivation occurred early after ICU admission and was associated with longer ICU length-of-stay.
Conclusions: While in the ICU, critically ill patients with COVID-19 are prone to develop reactivations due to various types of herpesviruses.

1. Introduction

Reactivation of herpesviruses is common in critically ill patients, even in the absence of pre-existing immunodeficiency [1–4]. Risk factors include prolonged invasive mechanical ventilation, intensive care unit (ICU) length-of-stay, severity of illness, and use of anti-inflammatory drugs [1–4]. All of these conditions are frequently met in critically ill patients with severe Coronavirus disease-19 (COVID-19). To date, there has been no report on concomitant reactivation of herpesviruses after severe acute respiratory syndrome – coronavirus 2 (SARS-Cov-2) infection.

Our aims in this study were to report the incidences of Epstein–Barr virus (EBV), cytomegalovirus (CMV), and human-herpes virus-6 (HHV-6) viremia in critically ill patients with COVID-19 and to assess their association with clinical outcome.

2. Methods

2.1. Patients

In this monocentric retrospective study, we included all patients admitted for severe acute respiratory syndrome-coronavirus 2 (SARS-Cov-2) infection between March 16th and August 6th, 2020 in our 10-bed ICU (Unit C, Intensive Care department, "Centre hospitalier universitaire de Lille", France). All patients were diagnosed with SARS-Cov-2 infection, according to the World Health Organisation criteria [5]. Nasopharyngeal and throat swab samples were obtained at admission from all patients, who were tested, as previously described [6], using real-time reverse transcriptase-polymerase chain reaction assays to identify SARS-CoV-2 infection [6].

Blood EBV DNA, CMV DNA and occasionally HHV-6 DNA are routinely quantified in our ICU for patients considered at high risk of reactivation. Critically ill patients with COVID-19 were hypothesised as belonging to this category, and were therefore monitored for EBV, CMV and HHV-6 reactivations until ICU discharge or death.

2.2. Viral DNAemia detection and serology

EBV, CMV, and HHV6 DNA detections were performed with quantitative PCR in whole blood by using AltoStar® EBV PCR Kit 1.5, AltoStar® CMV PCR Kit 1.5, and AltoStar® HHV-6 PCR Kit 1.5 (Altona Diagnostics, Germany). Viral DNAemia detection was scheduled to be performed twice a week during ICU stay.

CMV and EBV serology testing was performed to determine the serological status of each virus. IgG and IgM antibodies were measured by chemiluminescence immunoassays (LIAISON CMV

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Table 1
Baseline demographic and clinical patient characteristics.

Patient characteristics (n = 34)	
Median age – years (range)	58 (26–81)
Male sex – no. (%)	25 (73)
Mean BMI – kg/m^2 (range)	31.2 (24.8–42.9)
SAPS-II – median (range)	55 (26–80)
Median ICU length-of-stay – days (range)	12.5 (4–58)
Co-existing conditions	
Arterial hypertension – no. (%)	16 (47)
Diabetes – no. (%)	11 (32)
Dyslipidemia – no. (%)	13 (38)
Myocardiopathy – no. (%)	3 (9)
History of cancer – no. (%)	2 (6)
Immunodeficiency – no. (%)	2 (6)
Ventilatory support	
HFNC and / or NIV – no. (%)	4 (12)
IMV – no. (%)	30 (88)
IMV and ECMO – no. (%)	6 (18)
Specific COVID-19 treatment	
Lopinavir–Ritonavir and Interferon – no. (%)	1 (3)
Remdesivir – no. (%)	3 (9)
Hydroxychloroquine – no. (%)	1 (3)
Tocilizumab – no. (%)	1 (3)
Corticosteroid treatment[a] – no. (%)	30 (88)
Median delay between ICU admission and corticosteroid initiation – days (range)	3 (0–18)
Patient outcome (as of August 17th, 2020)	
ICU discharge – no. (%)	28 (82)
Death in the ICU – no. (%)	6 (18)

BMI: body mass index; CMV: cytomegalovirus; EBV: Epstein-Barr virus; ECMO: extracorporeal membrane oxygenation; HFNC: high-flow nasal cannula; HHV-6: human herpes virus-6; ICU: intensive care unit; IMV: invasive mechanical ventilation; NIV: non-invasive ventilation; SAPS-II: simplified acute physiology score-II.

[a] Corticosteroid treatment consisted in methylprednisolone 1 mg/kg/day for 10 days or dexamethasone 20 mg/day for 5 days followed by 10 mg/day for 5 days.

IgG II and IgM II, LIAISON EBV IgM, VCA IgG, EBNA IgG, DiaSorin, Italy).

2.3. Data collection

A trained team of physicians reviewed and collected epidemiological data, past medical history, treatments, clinical and biological data, and outcomes for all consecutive patients from their admission until August 17th, 2020. This observational study was based on medical records, in strict compliance with the French reference methodology MR-004, established by the French National Commission on Informatics and Liberties, and approved by the institutional data protection authority of CHU de Lille.

2.4. Statistical analysis

All results were expressed as median (range) for continuous variables and as frequency (percentage) for categorical variables. Continuous variables were compared between groups with Welch's t-test. Statistical analysis was performed using XLSTAT (Microsoft Corporation, Redmond, USA).

3. Results

3.1. Patient cohort

From March 16th to August 6th, 2020, 34 patients were admitted in our ICU for SARS-Cov-2-associated acute respiratory failure. Baseline demographic and clinical characteristics are listed in the Table 1. Median age was 58 years (range 26–81).

Acute respiratory failure was managed with high-flow nasal cannula and/or non-invasive ventilation in 4/34 cases (12%). Thirty patients (88%) required invasive mechanical ventilation (IMV) and 6/34 patients (18%) required extracorporeal membrane oxygenation in addition to IMV.

3.2. DNAemia detection

DNAemia detection for EBV was performed on average 3.8 times (range 1–15) per patient during ICU stay. EBV was detected at least once in the blood of 28/34 patients (82%) (Table 2). EBV DNA detection was positive, but not quantifiable in 9/28 patients (32%) and quantifiable at least once in the 19 other patients (68%). Median viral load in blood of patients with quantifiable EBV replication was 8,648 IU/mL. Median delay between ICU admission and initial EBV DNA detection was 4 days (range 0–20). Median duration of positive blood EBV DNA detection while in the ICU was 7 days (range 1–54), (Table 2).

DNAemia detection for CMV was performed on average 3.7 times (range 1–15) per patient during ICU stay. CMV DNA was detected at least once in the blood of 5/34 patients (15%) (Table 2). CMV DNA detection was positive, but not quantifiable in 1/5 patients (20%) and quantifiable in the 4 other patients, with a median viral load of 4,930 IU/mL. Median delay between ICU admission and first CMV DNA detection was 12 days (range 1–16). Median duration of positive blood CMV DNA detection while in the ICU was 15 days (range 1–54) (Table 2).

DNAemia detection for HHV-6 was performed on average 3.4 times (range 1–14) per patient during ICU stay. HHV-6 DNA was detected at least once in the blood of 7/32 patients (22%) (Table 2). Viral load was not quantifiable in 6/7 patients (85%) and low in one patient (15%). Median delay between ICU admission and initial HHV-6 DNA detection was 12 days (range 8–19). Median duration of positive blood HHV-6 DNA detection while in the ICU was one day (range 1–4) (Table 2).

3.3. Co-occurrence of DNAemia detections

Five patients in the cohort (15%) presented no viral DNAemia detection while in the ICU. Twenty patients (59%) were positive for one virus (EBV in 19 cases and HHV-6 in one case), 7/34 patients (20%) were positive for 2 viruses (EBV and CMV in 3 cases, EBV and HHV-6 in 4 cases) and 2/34 patients (6%) were positive for the 3 viruses.

3.4. EBV and CMV serology

EBV serology was tested in 13 patients with positive EBV DNAemia. All 13 serology test results were consistent with EBV reactivation and not with primary EBV infection [7].

CMV serology was tested in 4 patients among the 5 patients with positive CMV DNAemia, and was consistent with prior CMV infection. CMV serological status was also tested in 7 patients who did not present CMV viremia while in the ICU. Five patients (71%) were seropositive for CMV whereas 2/7 patients (29%) were seronegative.

3.5. Association of viremia with clinical outcomes

Among the 5 patients who presented with CMV reactivation, three received anti-CMV treatment (ganciclovir in 2 cases, valganciclovir in one) and were treated successfully.

No patient received anti-EBV or anti-HHV-6 treatment.

Six patients in the cohort (18%) died while in the ICU. Two of them (33%) had not developed any viral reactivation, 3/6 (50%) had isolated blood EBV reactivation, and 1/6 (17%) had developed both CMV and EBV reactivation. We found no association between ICU mortality and EBV, CMV and HHV-6 reactivation.

Table 2
EBV, CMV and HHV-6 blood replication.

EBV Blood DNA detection – No. positive / No. tested (%)	28/34 (82)
Positive but not quantifiable viral load – No. of patients / No. positive for EBV detection (%)	9/28 (32)
Quantifiable viral load – No. of patients / No. positive for EBV detection (%)	19/28 (68)
Median blood viral load in patients with quantifiable EBV replication – IU /mL (range)	8,648 (1,654 – 242,674)
Median delay between ICU admission and first EBV DNA detection – days (range)	4 (0–20)
Median duration of EBV viremia in the ICU – days (range)	7 (1–54)
CMV Blood DNA detection – No. positive / No. tested (%)	5/34 (15)
Positive but not quantifiable viral load – No. of patients / No. positive for CMV detection (%)	1/5 (20)
Quantifiable viral load – No. of patients / No. positive for CMV detection (%)	4/5 (80)
Median blood viral load in patients with quantifiable CMV replication – IU /mL (range)	4,930 (805 – 32,221)
Median delay between ICU admission and first CMV DNA detection – days (range)	12 (1–16)
Median duration of CMV viremia in the ICU – days (range)	15 (1–54)
HHV-6 Blood DNA detection – No. positive / No. tested (%)	7/32 (22)
Positive but not quantifiable viral load – No. of patients / No. positive for HHV-6 detection (%)	6/7 (86)
Quantifiable viral load – No. of patients / No. positive for HHV-6 detection (%)	1/7 (14)
Blood viral load in patient with quantifiable HHV-6 replication – IU /mL	11,430
Median delay between ICU admission and fist HHV-6 DNA detection – days (range)	12 (8–19)
Median duration of HHV-6 viremia in the ICU – days (range)	1 (1–4)

CMV: cytomegalovirus; EBV: Epstein–Barr virus; HHV-6: human herpes virus-6; ICU: intensive care unit.

EBV reactivation was associated with longer median ICU length-of-stay (15 days vs. 8 days, $P < 0.05$). CMV and HHV-6 reactivations were not associated with a significantly longer median ICU length-of-stay (27 days vs. 12 days, $P = 0.11$ for CMV, 16 days vs. 12 days, $P = 0.91$ for HHV-6).

Regarding disease severity, EBV reactivation and CMV reactivation were not significantly associated with higher mean simplified acute physiology score (SAPS)-II (55 vs. 33, $P = 0.06$ for EBV, 61 vs. 51 for CMV, $P = 0.40$). We found no association between HHV-6 reactivation and SAPS-II.

4. Discussion

In this cohort of critically ill patients with COVID-19, an 85% majority developed EBV, CMV and/or HHV-6 viremia while in the ICU. EBV was detected in blood samples from 82% of patients. This proportion is higher than the frequencies reported in septic-shock patients (35%–71%) [1–4] and in ICU-patients with sepsis due to community-acquired pneumonia (CAP) (37%) [8] We found EBV reactivation to occur early after ICU admission, in agreement with previous observations in cases of sepsis and CAP [3,4,8].

CMV and HHV-6 were detected in blood samples from 15% and 22% of COVID-19 patients, respectively. These figures are close to the frequencies reported in larger studies for septic-shock patients (18–40% for CMV and 10–24% for HHV-6) [1,3,4,9–12].

One limitation of our study is the lack of serological data for each of the viruses in the whole patient sample. EBV serology was tested in 13 patients with positive EBV DNAemia, and was consistent with prior EBV infection. CMV serology was tested in 4 patients with positive CMV DNAemia, and was similarly consistent with prior CMV infection. CMV serology was also tested in 7 patients with no CMV viremia during ICU stay, and was negative for 2/7 patients (29%). Of note, CMV reactivation cannot occur among seronegative patients. As a result, the percentage of CMV reactivation is higher than the proportion of patients with positive CMV DNAemia detection.

We found EBV to be significantly associated with longer ICU length-of-stay, which is consistent with previous reports [1,2]. We did not find other significant association between EBV, CMV and HHV-6 reactivations and clinical outcomes. This is partly due to the small size of our cohort, which is another limitation of our study.

A third limitation is the absence of a comparative population with mild, moderate, or no COVID-19. We could therefore not assess whether SARS-Cov-2 infection and/or "ICU-acquired immunosuppression" was the main driver for herpesvirus reactivation.

All in all, our results show that critically ill patients with SARS-Cov-2 infection are prone to develop EBV, CMV and HHV-6 reactivations while in the ICU. Large-scale studies are necessary in the COVID-19 domain to identify risk factors for herpesviruses reactivations, their association with morbidity and mortality, and their relationship with host immune response.

Data

This observational study was based on medical records, in strict compliance with the French reference methodology MR-004, established by the French National Commission on Informatics and Liberties, and approved by the institutional data protection authority of CHU de Lille. Patient confidentiality was protected by assigning an anonymous identification code, and the electronic data were stored in a locked, password-protected computer.

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Author credit statement

Arthur Simonnet and Ilka Engelmann contributed to the conceptualisation, methodology, data curation, and writing of the original draft preparation; Anne-Sophie Moreau, to the conceptualisation, writing of the original draft preparation, and supervision; Bruno Garcia, to the data curation, and writing – reviewing and editing; Sophie Six, Ahmed El Kalioubie, and Laurent Robriquet, to the conceptualisation, and writing – reviewing and editing; Didier Hober, to the conceptualisation, writing – reviewing and editing, and supervision; and Mercé Jourdain, to the conceptualisation, methodology, writing – reviewing and editing, and supervision. The authors are grateful to Pr Julie Kerr-Conte for correcting the manuscript and to the reviewers for constructive, relevant and highly valuable remarks

Disclosure of interest

The authors declare that they have no competing interest.

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