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The reactivation of herpesviruses in severe COVID-19; a retrospective analysis of a critical care cohort

SARS-CoV-2 and its association with secondary infections is well described, however the reactivation of latent viral infections in the setting of COVID-19 is becoming increasingly recognised. The *Herpesviridae* family in particular has been a focus of much review, the five most commonly implicated including cytomegalovirus (CMV), Ebstein-Barr virus (EBV), varicella zoster virus (VZV) and herpes simplex virus (HSV) 1 and 2 (Grinde, 2013). We read with interest a study by *Niitsu et al* that describes the reactivation of CMV among a critically unwell cohort of patients requiring mechanical ventilation (Niitsu et al., 2021). The authors note lymphopaenia and longer ventilation times among patients with CMV infection, results that are echoed in similar studies (Gatto et al., 2022). However, there is a paucity of data on the reactivation of other herpesviruses in the setting of SARS-CoV-2 infection, the sequelae of which are thought to play a role in the pathophysiology of long-COVID (Gold et al.,). Here, we discuss the frequency of CMV, EBV, VZV and HSV 1 and 2 reactivation among patients with COVID-19 requiring critical care.

A retrospective analysis of all patients with COVID-19 requiring admission to the intensive care (ICU) and high dependency unit (HDU) at our institution from March 2020 until December 2021 was performed as part of the Anticipate Study (Avramovic et al., 2021). Baseline patient characteristics and virology were collected from the institution's electronic patient record system. All patients were confirmed positive for SARS-CoV-2 by reverse transcriptase polymerase chain reaction (RT PCR). Both EBV and CMV infection were defined by the detection of DNA in blood using quantitative PCR analysis. VZV and HSV infection was defined by the presence of DNA on a viral swab of a skin lesion. The nadir lymphocyte count and peak c-reactive protein (CRP) for each patient was collected. All data was analysed using STATA version 17.0. Categorical variables were analysed using chi-square and the student *t*-test was used to compare means.

From the beginning of the pandemic to December 2021 there were 295 patients with COVID-19 that required management in the ICU/HDU, of which 40% (117/295) were female with a mean age was 55 years (standard deviation (SD) 15.1 years). Of the 67 patients who were screened for CMV infection, 22.3% (15/67) were positive. Though not tested in all patients, there were no CMV infections with a baseline negative CMV IgG. There was no association between death and CMV infection (40% (6/15) mortality, $p = 0.807$), however a significant relationship between CMV infection and mechanical ventilation was noted ($p < 0.0001$). Of those who required ventilation, significantly longer time was spent on a ventilator than those who tested negative for CMV infection (mean 879 h compared with 301 h, $p < 0.0001$).

EBV infection was detected in 26% (7/27) of the 27 patients that were tested and there were no infections in patients that were EBV IgG negative at baseline. There was no relationship between EBV infection and death ($p = 0.853$) or invasive ventilation ($p = 0.098$). Collectively, 9% (26/296) of the cohort tested positive for HSV-1 (92%; 24/26) and VZV (8%; 2/26) on viral swabs of a skin lesion. Of note, a clinical diagnosis of herpes zoster from a dermatomal rash was not possible to clarify from the electronic patient record.

Each patient's nadir lymphocyte count was used to examine the potential link between lymphopaenia and viral reactivation. One outlier with chronic lymphocytic leukaemia (CLL) was removed from this analysis with a nadir lymphocyte count 18 standard deviations from the mean. Among the revised cohort ($n = 294$), the mean nadir-lymphocyte count was 0.62 $x10^9$/L (SD 0.43, 95% 0.58 – 0.69). While the mean nadir-lymphocyte count was in fact higher in those with CMV infection than those who tested negative, this was not statistically significant ($p = 0.684$). Patients with EBV infection had a lower lymphocyte count than those who tested negative, a trend also seen among those with a positive viral swab for HSV and VZV (see Table 1.). Similarly, the peak CRP was measured for each patient as a surrogate marker for inflammation severity and IL-6 (Herold et al., 2020). We found that the mean peak-CRP was higher in those with EBV and CMV infection and also those that tested positive for VZV/HSV on viral swab (see Table 2).

While this study is limited in its retrospective design, we have found that the reactivation of CMV (22.3%), EBV (26%), and HSV/VZV (9%) in this cohort is in line with what has previously been documented (Saade et al., 2021). CMV infection in particular was associated with invasive ventilation and longer ventilation times, as previously described (Niitsu et al., 2021; Gatto et al., 2022). However, contrary to results from other studies, there was no association with lymphopaenia and CMV infection, although the measurement of a single nadir lymphocyte count likely underestimates the time spent in a lymphopenic state. Furthermore, while not fully elucidated, the reactivation of latent viral infections is certainly multifactorial. The cytokine storm caused by SARS-CoV-2 infection is thought to induce herpesvirus reactivation via TNF-alpha and IL-6 among others, while the transcripts of SARS-CoV-2 are thought to interact with herpesvirus elements directly (Chen et al., 2022).

Viral reactivation is not exclusive to SARS-CoV-2 infection, with herpesvirus reactivation documented prior to the onset of the pandemic in critically unwell patients in the ICU (Coşkun et al., 2017). As seen in our study, it is often associated with higher CRP values, mechanical ventilation and lymphopaenia. However, recent data from a non-critical care cohort has implicated EBV reactivation in the pathophysiology of long-COVID. In a study by *Gold et al* that investigated the prevalence of EBV reactivation in a long-COVID cohort, as defined by a positive titre for EBV EA-D IgG or EBV VCA IgM, 66.7% of long-term long-COVID patients were found to be positive for EBV reactivation (Gold et al., 2021). With many of these patients documented as asymptomatic to

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Table 1
Lymphocyte analysis in herpesvirus reactivation.

	Number of patients*	Nadir lymphocyte count (mean) (x10^9/L)	95% Confidence Interval	p-value**
CMV DNA positive	14/66	0.535	0.414–0.587	0.684
CMV DNA negative	52/66	0.492	0.390–0.593	
EBV DNA positive	7/26	0.476	0.25–0.702	0.646
EBV DNA negative	19/26	0.548	0.363–0.735	
Viral swab positive***	26/294	0.505	0.364–0.645	0.143
No viral swab	268/294	0.634	0.581–0.686	

* One outlier removed from lymphocyte analysis.
** Two-sample t-test to compare means.
*** HSV-1 and VZV positive viral swabs combined.

Table 2
CRP analysis in herpesvirus reactivation.

	Number of patients	Peak CRP (mg/L)	95% Confidence Interval	p-value*
CMV DNA positive	15/66	292	213–371	0.206
CMV DNA negative	52/66	244	209–279	
EBV DNA positive	7/26	313	244–382	0.078
EBV DNA negative	19/26	214	150–278	
Viral swab positive**	26/295	215	168–262	0.917
No viral swab	268/295	212	198–227	

* Two-sample t-test to compare means.
** HSV-1 and VZV positive viral swabs combined.

their initial infection, it strengthens the argument that SARS-CoV-2 infection itself plays a role in viral reactivation, independent of disease severity.

In conclusion, we believe that the reactivation of herpesviruses in the setting of SARS-CoV-2 infection warrants further research, with the virus itself capable of stimulating other infections and potentially contribute to an enhanced disease severity and a protracted symptom course, as seen in long-COVID.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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