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Original article

Herpes simplex virus reactivation among severe COVID-19 patients: to treat or not to treat?

N. Coosemans[1], J. Janssen[2], J. Klein[2], T. Schepens[3,4,5], W. Verbrugghe[5], P. Jorens[2,5]

[1]Department of Anesthesiology, Antwerp University Hospital, Drie Eikenstraat 655, 2650 Edegem, Belgium; [2]Faculty of Medicine and Health Sciences, University of Antwerp, Universiteitsplein 1, 2610 Antwerp, Belgium; [3]Department of Intensive Care Medicine, Ghent University Hospital, C. Heymanslaan 10, 9000 Ghent, Belgium; [4]Department of Internal Medicine and Pediatrics, Ghent University, C. Heymanslaan 10, 9000 Ghent, Belgium; [5]Department of Intensive Care Medicine, Antwerp University Hospital, University of Antwerp, LEMP, Drie Eikenstraat 655, 2650 Edegem, Belgium

Corresponding author: Coosemans N., Department of Anesthesiology, Antwerp University Hospital, Drie Eikenstraat 655, 2650 Edegem, Belgium. E-mail: nathalie.coosemans@gmail.com

Abstract

Background: **Herpes simplex virus type 1 (HSV-1) reactivation in the airways is a common finding among patients admitted to the intensive care unit and has been more recently been reported in critically ill COVID-19 patients. Evidence suggests that HSV-1 reactivation in critically ill patients may be associated with higher morbidity and mortality rates. However, there is conflicting data about whether treatment with acyclovir impacts outcomes.**

Objectives: **The primary aim of this study is to assess whether acyclovir improves survival in critically ill COVID-19 patients with concomitant HSV-1 reactivation. Additionally, we explore the effect of acyclovir on cardiorespiratory instability, biochemical markers of inflammation and renal function. Incidence, potential risk factors and outcomes of HSV-1 reactivation in COVID-19 ICU patients are studied last.**

Methods: **A retrospective single-center cohort study set in a Belgian tertiary-care university hospital. All COVID-19 patients admitted to the ICU between March 1st, 2020, and April 15th, 2021, and were tested for HSV-1 using real-time PCR in airway samples were included for analysis. The administration of acyclovir for patients with HSV-1 reactivation was not randomized. Mortality and various markers of morbidity (cardiorespiratory instability, biochemical markers of inflammation, and renal function) were compared between patients that had received acyclovir and those that had not. Secondary outcome measures were respiratory and inflammatory markers of disease severity.**

Results: **34.7% (42/121) of patients had HSV-1 reactivation, of which 67% (28/42) received acyclovir. ICU mortality was 36% (n = 10) in the acyclovir group versus 0% in the untreated group. Multivariate analysis resulted in OR 3.82 (95% CI 1.37 – 10.68) for ICU mortality in the treated group. Patients treated with acyclovir had a longer length of stay (41.8 vs. 26.8 days, p = .018), longer duration of invasive mechanical ventilation (33.4 vs. 21.8 days, p = .050), and lower PaO2/FiO2 ratio (59.9 vs. 73.4 mmHg, p = .008).**

Conclusions: **The role of acyclovir in patients with HSV-1 reactivation in the ICU remains controversial. According to this study, respiratory HSV-1 reactivation for this specific patient group might be better left untreated. Treatment selection bias, however, could not be fully excluded.**

Keywords: **COVID-19, SARS-Cov-2, herpes simplex virus, HSV-1, acyclovir, intensive care.**

Disclaimer: This paper serves as a master thesis for graduation of N. Coosemans, MD in Anesthesia and Intensive Care and has been submitted for presentation on the BeSARPP Graduation Day 2023.

The Ethics Committee of the Antwerp University Hospital, chaired by Prof. dr. G. Ieven, granted approval of this study on February 15th, 2021 (EC 21/06/100), including a waiver of informed consent, as all data were gathered retrospectively, with EDGE number 1650.

Introduction

Following primary infection, HSV-1 establishes latency in the sensory ganglia neurons, where it can reactivate in response to various stimuli, including fever and critical illness[1,2]. While HSV-1 infection of the lower respiratory tract in the general population is uncommon, it has been frequently detected in samples of immunocompromised and critically ill patients[2-6]. Although the majority of HSV-1 reactivation cases have an asymptomatic to mild course, severe complications such as pneumonia, encephalitis, and disseminated infection have been documented[1,2,7].

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the virus that causes COVID-19, has been associated with lymphopenia,[8,9] and can trigger a cytokine storm.10-12 The resulting immunosuppressive state may encourage reactivation of latent viral infections, such as HSV-1[10,13,14]. The incidence of HSV-1 reactivation is significantly higher among COVID-19 patients hospitalized in the ICU compared to patients without COVID-19[13-16]. Studies indicate that the proportion of critically ill COVID-19 patients with detectable HSV-1 DNA in their bronchoalveolar lavage samples ranges from 27% to 83%[3,9,14-19]. Hence, it has been hypothesized that COVID-19 infection might be a risk factor for HSV-1 reactivation and subsequent pulmonary infection of HSV-1[16,17,20].

Despite the high prevalence of HSV-1 reactivation in critically ill patients, the clinical implications and optimal management of this condition remain unclear[7,21-24]. Observational data regarding the potential benefits of antiviral agents such as acyclovir are contradictory[4-7,13]. A meta-analysis from Hagel et al. suggests that antiviral therapy is associated with lower hospital mortality and 30-day mortality, albeit with low quality of evidence[24]. HSV-1 reactivation in severe COVID-19 patients may be associated with increased morbidity and mortality;[15,18] however, no studies have examined the effects of acyclovir in this specific patient population thus far.

Objectives

Ethical approval

The study, publicly registered under EDGE number 1650, was conducted in accordance with the amended Declaration of Helsinki (version October 2013). The Ethics Committee of the Antwerp University Hospital granted approval on February 15th, 2021 (EC 21/06/100), including a waiver of informed consent, as all data were gathered retrospectively. Pseudonymization was achieved by indexing patients by their unique patient identification numbers from the electronic medical records.

Study population

All adult patients admitted to the Intensive Care Unit of the Antwerp University Hospital between March 1st, 2020, and April 15th, 2021, with COVID-19 associated acute respiratory failure as the main reason for admission, were identified. SARS-CoV-2 infection was confirmed in all cases by polymerase chain reaction (PCR) with cycle threshold values below 32. Exclusion criteria were patients under 18 years of age on the day of admission or the absence of severe respiratory failure (i.e., patients with asymptomatic or mild SARS-CoV-2 infection). From this population, eligible patients were tested for HSV-1 in at least one respiratory sample (defined as noninvasive to more invasive and lower respiratory tract samples; sputum, nasopharyngeal, endotracheal, or bronchial aspirate, or bronchoalveolar lavage). HSV-1 reactivation was defined in patients with at least one positive PCR result for HSV-1. Two groups were defined: an intervention group receiving treatment with acyclovir and a group not treated with acyclovir labeled as the control group. All patients received acyclovir for five days or longer intravenously at 10 mg.kg-1 three times daily.

Study design

This retrospective single-center, observational study is reported according to the 'Strengthening The Reporting of Observational Studies in Epidemiology' (STROBE) statement[3].

The records (Cegeka C2M), as well as the ICU Patient Data Management System (iMD-Soft MetaVision), were consulted for patient demographic characteristics. The patient's history was reviewed for known risk factors for HSV-1 reactivation, such as diabetes, chronic heart or renal failure, and the chronic use of immunosuppressive drugs or steroids[4,6,26]. As markers of disease severity, the SAPS-3 (Simplified Acute Physiology Score) and the SOFA (Sequential Organ Failure Assessment) score were considered[27,28]. The following data were considered relevant: total duration of ventilation and ICU stay, P/F ratio, ICU and in-hospital mortality, serum inflammatory markers (CRP, IL-6, lymphocyte count), and the administration of certain drugs during the ICU period (acyclovir, corticosteroids, vasopressors, and inotropic agents).

There was no protocol in place to determine which patients warranted treatment with antiviral agents, and the decision to start a patient on acyclovir was left to the discretion of the treating

critical care physician. To correct for bias, wherein more severely ill patients would be more likely to receive antiviral treatment, a multivariate analysis to correct for known indicators of disease severity, such as maximal SOFA score and parameters of kidney function, was performed.

Outcome measures

The primary outcome measure of this study is mortality, defined by overall mortality in the ICU, mortality within the first 28 days after ICU admission, and in-hospital mortality. Secondary outcome measures are markers of respiratory insufficiency and hemodynamic instability, defined by the lowest PaO2/FiO2 ratio, number of days of invasive mechanical ventilation (IMV) or high-flow nasal oxygen (HFNO), and number of days of vasopressor or inotropic support. Other secondary outcome measures of interest are certain biochemical parameters acting as a surrogate for inflammation and the immune response, such as C-reactive protein (CRP), interleukin-6 (IL-6), ferritin, and the presence of lymphopenia.

Statistical analysis

All analyses were performed with SPSS version 28 (SPSS Inc. Chicago, IL, USA). Differences between groups (treatment versus control, HSV-1 positive versus HSV-1 negative) were assessed using several statistical tests: the chi-square ($\chi 2$) test was used to compare categorical variables, and the data was reported as the number with percentage, but when expected values in the contingency table for a given variable fell below five, Fisher's exact test was used instead. The Shapiro-Wilk test was used to check the normal distribution of continuous variables. When data were not normally distributed, the Mann–Whitney U test was performed instead of the continuous samples t test, and the variable was reported as mean with range. A two-sided p value, or alpha, less than .05 was considered statistically significant.

Univariate and multivariate COX regression analyses were performed for the treatment groups and the individual other risk factors. The risk factors significantly associated with mortality in the univariate regression were included in a forward conditional multiple cox regression model to control for potential confounding variables.

To address potential selection bias in treatment assignments, a propensity score-matched cohort design was used. The propensity score predicted the probability of receiving acyclovir treatment based on several factors, including the history of chronic kidney disease, the need for renal replacement therapy, the severity of illness scores (SAPS-3 score on admission and maximum SOFA score), and immunosuppressive therapy. All variables were entered in a logistic regression model with acyclovir as the dependent variable, hereby assigning a propensity score to each patient. This propensity score was used in regression analysis for both ICU and in-hospital mortality. The quintiles of these propensity scores were then used as strata in survival analysis using cox proportional hazards.

Results

Study population

One hundred ninety-nine patients were assessed for eligibility, of which 61% (121/199) had been tested for the presence of HSV-1. Figure 1 shows the flowchart of the study population. Not visualized in this diagram are the seventeen patients that were

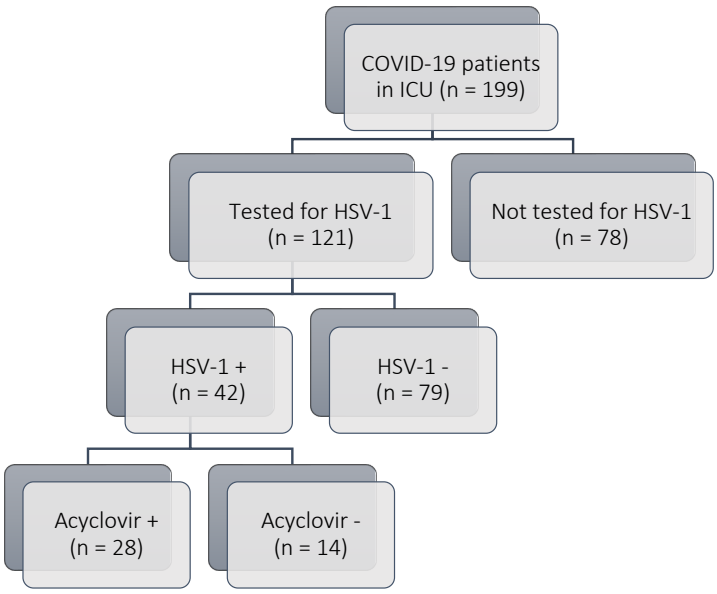

Fig. 1 — Study Flow Diagram.

readmitted to the ICU, both during the study period and for the same reason (COVID-19 associated ARDS), from whom we combined the relevant data with the first admission and analyzed them as if they had had a single ICU stay.

Table I summarizes patient baseline characteristics of all patients tested for HSV-1 in at least one upper or lower respiratory sample. The mean age was 60.3 years (interquartile range (IQR), 52 – 70), and two-thirds (81/121, 66.9%) of them were male. 34.7% (42/121) of patients had HSV-1 reactivation. Both groups were comparable in age, gender, and comorbidities, except for a higher percentage of chronic renal failure in the HSV-1 positive group (17% vs. 4%, p = .031). Of the 42 patients that tested positive for HSV-1, a total of 28 patients received acyclovir, and 14 did not. The patient characteristics in the intervention versus control group, shown in Table II, were comparable in age, gender, weight, BMI, and disease severity (SAPS-3, SOFA-score at the time of ICU admission). Both groups had similar pre-existing comorbidities.

Primary endpoint: mortality

The ICU mortality was 0% in the control group and 36% in the acyclovir group (n = 10), p = .017 (see Figure 2). One extra patient died in the

Table I. — Demographics of the study population tested for HSV-1.

	Total (n = 121)	HSV-1 + (n = 42)	HSV-1 – (n = 79)	P value
Patient characteristics				
Male gender	81 (67%)	31 (74%)	50 (63%)	.311
Age (years)	60.3 (27-88)	59.7 (29-81)	60.6 (27-88)	.498
Weight (kg)	86.6 (53-173)	84.2 (53-147)	87.9 (59-173)	.305
BMI ($kg.m^{-2}$)	28.9 (19.0-57.0)	28.4 (20.6-42.2)	29.2 (19.0-57.0)	.838
Main risk factors on admission				
Diabetes mellitus	13 (11%)	4 (10%)	9 (11%)	1
Immunosuppressant's use	18 (15%)	9 (21%)	9 (11%)	.180
Chemotherapy	4 (3%)	0 (0%)	4 (5%)	.297
Radiotherapy	4 (3%)	0 (0%)	4 (5%)	.297
Chronic heart failure	10 (8%)	1 (2%)	9 (11%)	.162
COPD	8 (7%)	4 (10%)	4 (5%)	.446
Chronic renal failure	10 (8%)	7 (17%)	3 (4%)	.031**
Arterial hypertension	62 (51%)	23 (55%)	39 (49%)	.703
Disease severity				
ICU mortality	31 (26%)	10 (24%)	21 (27%)	.829
In-hospital mortality	33 (27%)	11 (26%)	22 (28%)	.832
Mortality on day 28	18 (15%)	3 (7%)	15 (19%)	.109
SAPS-3 on admission	55.1 (37-79) 1*	56.4 (42-77)	54.3 (37-79) 1*	.276
SOFA on admission	9.6 (2-20) 9*	10.5 (2-19) 3*	9.2 (2-20) 6*	.102
SOFA max	13.3 (3-21) 9*	14.2 (5-20) 3*	12.9 (3-21) 6*	.015**
Length of stay (days)	28.9 (1-84)	36.8 (8-70)	24.7 (1-84)	<.001**
Total HFNO duration (days)	8.4 (0-37)	9.6 (0-37)	7.8 (0-32)	.195
Total IMV duration (days)	24.8 (0-83)	29.8 (0-66)	22.2 (0-83)	.008**
Lowest P/F ratio	66.9 (5-158)	64.4 (30-158)	68.3 (5-155)	.092
ECMO/ECCO2R	24 (20%)	11 (26%)	13 (16%)	.235
Corticosteroid use in ICU	81 (67%)	29 (69%)	52 (66%)	.840
Need for vasopressors and inotropics				
Epinephrine (days)	.1 (0-4)	.1 (0-2)	.2 (0-4)	.589
Norepinephrine (days)	11.7 (0-48)	15.0 (0-38)	10.0 (0-48)	.005**
Vasopressin (days)	.2 (0-5)	.3 (0-3)	.2 (0-5)	.190
Dobutamine (days)	.2 (0-5)	.3 (0-5)	.1 (0-5)	.330
Milrinone (days)	.9 (0-34)	.1 (0-2)	1.4 (0-34)	.113

HSV-1 + and – indicate the patients that tested positive or negative, respectively, for Herpes simplex type 1 on a respiratory sample. Continuous variables are reported as mean with range between brackets. Categorical variables are reported as the number with percentage. *Number of missing records that were excluded from the analysis. **A two-sided p < .05 indicates statistical significance.

Table II. — Characteristics of patients with HSV-1 reactivation divided by treatment group.

	Total (n = 42)	Acyclovir + (n = 28)	Acyclovir – (n = 14)	P value
Patient characteristics				
Male gender	31 (74%)	20 (71%)	11 (79%)	.723
Age (years)	59.7 (29-81)	61.4 (45-81)	56.4 (29-76)	.219
Weight (kg)	84.2 (53-147)	84.6 (53-147)	83.4 (67-115)	.759
BMI ($kg.m^{-2}$)	28.4 (20.6-42.2)	28.3 (20.7-40.7)	28.6 (21.6-42.2)	.979
Main risk factors on admission				
Diabetes mellitus	4 (10%)	2 (7%)	2 (14%)	.590
Immunosuppressant's use	9 (21%)	5 (18%)	4 (7%)	.451
Chronic heart failure	1 (2%)	0 (0%)	1 (7%)	.333
COPD	4 (10%)	4 (14%)	0 (0%)	.283
Chronic renal failure	7 (17%)	3 (11%)	4 (29%)	.197
Arterial hypertension	23 (55%)	14 (50%)	9 (64%)	.515
Disease severity				
ICU mortality	10 (24%)	10 (36%)	0 (0%)	.017**
In-hospital mortality	11 (26%)	11 (39%)	0 (0%)	.007**
Mortality on day 28	3 (7%)	3 (11%)	0 (0%)	.539
SAPS-3 on admission	56.4 (42-77)	57.9 (42-77)	53.4 (42-71)	.112
SOFA on admission	10.5 (2-19)*	11.1 (2-19)*	9.4 (4-16)	.188
SOFA max	14.2 (5-20)*	14.6 (11-20)*	13.4 (5-18)	.298
Length of stay (days)	36.8 (8-70)	41.8 (14-70)	26.8 (8-60)	.018**
Total HFNO duration (days)	9.6 (0-37)	9.5 (0-37)	9.7 (4-20)	.799
Total IMV duration (days)	29.8 (0-66)	33.4 (0-66)	21.8 (0-45)	.050**
Lowest P/F ratio	64.4 (30-158)	59.9 (30-158)	73.4 (47-140)	.008**
ECMO/ECCO2R	11 (26%)	10 (36%)	1 (7%)	.067
Corticosteroid use in ICU	29 (69%)	22 (79%)	7 (50%)	.082
Need for vasopressors and inotropics				
Epinephrine (days)	.1 (0-2)	.1 (0-2)	.1 (0-1)	.976
Norepinephrine (days)	15.0 (0-38)	11.9 (0-35)	16.1 (0-38)	.125
Vasopressin (days)	.3 (0-3)	.3 (0-3)	.1 (0-2)	.491
Dobutamine (days)	.3 (0-5)	.4 (0-5)	.1 (0-2)	.656
Milrinone (days)	.1 (0-2)	.0 (0)	.1 (0-2)	.157

Acyclovir + and – indicates the patients that were treated with or without acyclovir, respectively. Continuous variables are reported as mean with range. Categorical variables are reported as frequency with percentage. *Three missing records were excluded from analysis. **A two-sided $p < .05$ indicates a significant difference between the treatment and control groups.

hospital after ICU discharge, totaling the total mortality in the acyclovir treatment group at 39% (n = 11), p = .007. Variables that were significantly associated with ICU mortality were creatinine levels, both at ICU admission and during the stay, and the presence of comorbidities such as chronic obstructive pulmonary disease (COPD) and arterial hypertension.

In a COX regression analysis, the survival time was defined as either the time to discharge or the time to death following admission to the ICU. A forward stepwise conditional multivariate model was used, which included all significant risk factors from the univariate analysis. The results showed that individuals treated with acyclovir had a relative risk for mortality in the ICU of 2.85 (95% CI 1.38 – 5.88, p = .005) compared to the control group. To account for possible treatment selection bias, a propensity score was included as a stratum in the multivariate analysis, which resulted in an odds ratio of 3.82 (95% confidence interval (CI) 1.37 – 10.68, p = .011) for ICU mortality in the treated group.

Secondary endpoints: markers of respiratory insufficiency and hemodynamic instability

95% (40/42) of patients received IMV through endotracheal intubation or tracheostomy for a mean duration of 31 days (SD 15.8). The patients treated with acyclovir were significantly longer

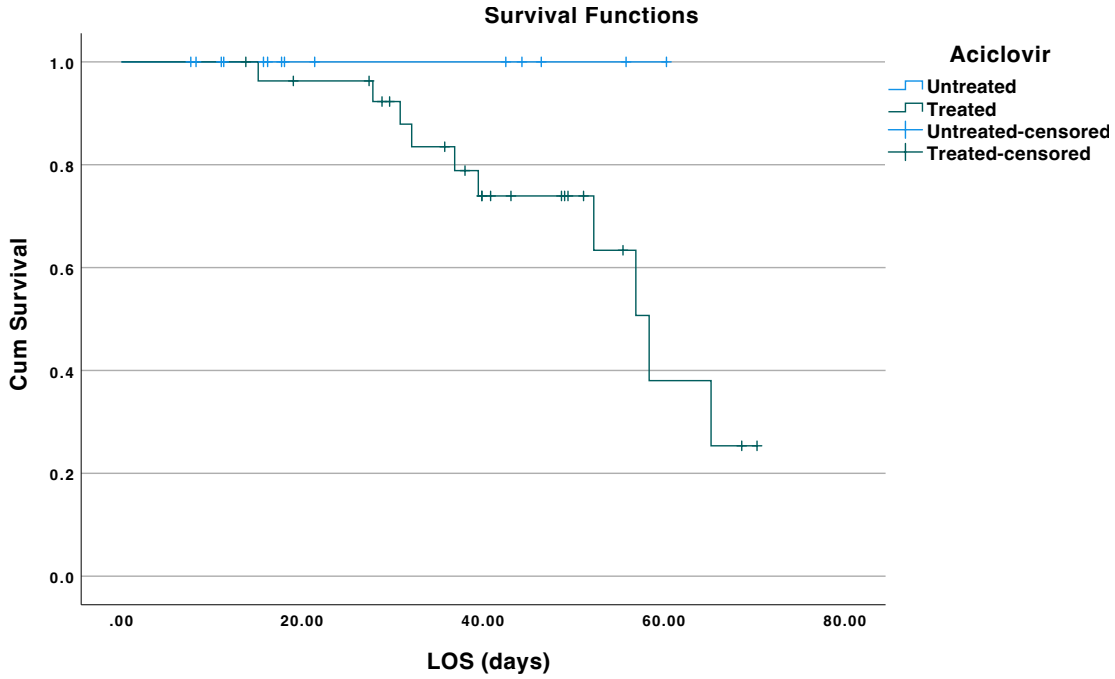

Fig. 2 — Kaplan-Meier survival analysis of patients treated with acyclovir versus without treatment.

mechanically ventilated (34.6 (SD 14.8) vs. 23.5 days (SD 15.7), p = .050).

A total of 36 patients received HFNO during their ICU stay. The six patients that didn't receive HFNO were all invasively mechanically ventilated and part of the acyclovir-treated group. The mean total HFNO duration was 9.6 days. There was no significant difference in HFNO duration between the acyclovir and the control groups (9.5 vs. 9.7 days, p = .799).

We recorded the lowest P/F ratio for every patient as a surrogate for ARDS severity. The mean lowest P/F ratio was 64.4 mmHg (SD 24.85, range 30 – 158) for all included patients combined. The control group had a significantly higher mean lowest P/F ratio compared to the acyclovir group (73.4 vs. 59.9 mmHg, p = .008).

One in four (26%, 11/42) patients was placed on extracorporeal membrane oxygenation (ECMO) or CO2 removal (ECCO2R) devices for a mean period of 30 days (range 15 to 60 days, SD 17.0). Even though all of these patients except one were treated with acyclovir, indicating a trend toward a higher percentage of ECMO use in the treated group, the difference was not statistically significant (10 (36%) vs. 1 (7%), p = .067).

Secondary endpoints: biochemical parameters of immune response and renal function

Table III shows various laboratory findings regarding the immune response and parameters of kidney function in COVID-19 patients with HSV-1 reactivation, divided by treatment group.

Both groups had comparable mean CRP levels on admission (treated versus control, 196.1 vs. 196.7 mg.l-1 , p = .894). The mean lymphocyte count on admission was similar (treated versus control, 8.3 vs. 11.6 E9.l-1, p = .060), as were the mean ferritin levels on admission (treated versus control, 1704 vs. 2620 µg.l-1, p = .052). and mean IL-6 levels (treated versus control, 257 vs. 149 pg.ml-1, p = .204). The highest values of these inflammatory markers were not significantly different between the two groups. However, a trend towards relative lymphopenia in the acyclovir-treated group could be observed (3.0 vs. 4.9 E9.l-1, p = .058).

There was no difference in creatinine level on admission with 1.07 mg.dl-1 (SD .72) in the acyclovir compared to 1.41 mg.dl-1 (SD 1.30) in the control group, p = .679, in the mean maximal creatinine value during ICU stay (treated versus control, 1.72 mg.dl-1 (SD 1.23) vs. 2.00 mg.dl-1 (SD 1.66), p = .926) or in the percentage of patients needing renal replacement therapy (RRT) (treated versus control, 25% vs. 29%, p = 1).

Outcome and potential risk factors of HSV-1 reactivation

One hundred twenty-one patients were separated into two groups, HSV-1 positive (n=42) and HSV-1 negative (n=79), to analyze outcomes and potential risk factors. As indicated in Table I, univariate analysis was conducted after collecting the necessary data on patient characteristics, primary risk factors on admission, disease severity, and clinical outcomes.

Regarding patient characteristics, including age, gender, weight, and body mass index, there was no significant difference between the two groups. Likewise, there was no significant

Table III. — Biochemical parameters of patients with HSV-1 reactivation divided by treatment group.

	Total (n = 42)	Acyclovir + (n = 28)	Acyclovir – (n = 14)	P value
Inflammatory markers on admission				
CRP ($mg.l^{-1}$)	196.3 (19.4-370.0)	196.1 (19.4-370)	196.7 (99.2-319.4)	.894
Lymphocyte count ($10^9.l^{-1}$)	9.4 (1.5-27.9)	8.3 (1.5-27.9)	11.6 (3.2-22.2)	.060
Ferritin ($\mu g.l^{-1}$)	1999.9 (342-4632) 11*	1704.2 (350-3073) 7*	2620.9 (342-4632) 4*	.052
IL-6 ($pg.ml^{-1}$)	226.7 (10-2560) 14*	257.8 (10-2560) 8*	149.0 (67-214) 6*	.204
Inflammatory markers extremes				
Highest CRP value ($mg.l^{-1}$)	350.0 (140.5-992.0)	336.6 (140.5-529.3)	376.9 (206.8-992.0)	.979
Lowest lymphocyte count ($10^9.l^{-1}$)	3.6 (.7-12.5)	3.0 (.7-7.2)	4.9 (1.5-12.5)	.058
Highest ferritin value ($\mu g.l^{-1}$)	3589.8 (94-14884) 1*	3665.8 (94-14884)	3425.9 (613-8179) 1*	.595
Highest IL-6 value ($pg.ml^{-1}$)	781.5 (17-18539) 7*	1028.7 (45.1-18539) 3*	163.7 (17-384) 4*	.391
Parameters of renal function				
Creatinine on admission ($mg.dl^{-1}$)	1.18 (.39-5.29)	1.07 (.39-4.32)	1.41 (.51-5.29)	.679
Highest creatinine ($mg.dl^{-1}$)	1.82 (.40-5.53)	1.72 (.40-5.18)	2.00 (.60-5.53)	.926
Need for RRT	11 (26%)	7 (25%)	4 (29%)	1

Acyclovir + and – indicates the patients were treated with or without acyclovir, respectively. The need for renal replacement therapy (RRT) is reported as a number with percentage between brackets. All other variables are presented as mean with range between brackets. *Number of missing records that were excluded for analysis.

difference between the two groups in terms of major risk factors at admission, such as diabetes mellitus, immunosuppressant use, chemotherapy, radiotherapy, chronic heart failure, COPD, arterial hypertension, and ICU mortality. However, there was a significant difference in the prevalence of chronic renal failure, with a higher proportion of HSV-1 positive patients having this comorbidity than HSV-1 negative patients (17% vs. 4%, p = .031).

There was no significant difference between the two groups in terms of illness severity and clinical outcomes, including ICU mortality, in-hospital mortality, and day 28 mortality. In addition, there was no significant difference in the SAPS-3 score on admission, the total duration of HFNO, the lowest P/F ratio, or the use of corticosteroids in the ICU.

However, HSV-1 positive patients had a significantly longer ICU length of stay compared to HSV-1 negative patients (36.8 vs. 24.7 days, p < .001) and a significantly longer overall IMV duration (29.8 vs. 22.2 days, p = .008). Furthermore, HSV-1 positive patients had a higher maximal SOFA score (14.2 vs. 12.9, p = .015) and required norepinephrine vasopressor support for a more extended time (15 vs. 10 days, p = .005).

Discussion

Effect on mortality

Long-standing controversy surrounds the effect of HSV-1 reactivation on mortality in critically ill patients without COVID-19, with a single randomized controlled trial indicating no impact on mortality, and a meta-analysis conversely suggesting a potential benefit of antiviral treatment[24,29]. However, a recent study conducted with COVID-19 patients found a significantly higher thirty day mortality rate in patients with an HSV-1 PCR positive result (57.4% vs. 33.5%, with n = 83 and p = .015)[15].

The presented study showed no difference in mortality when comparing HSV-1 positive and negative patients (24% vs. 27%, p = .829). ICU mortality in the acyclovir group is 36% (n = 10), compared to 0% in the control group, p = .017. Additionally, the COX regression analysis found a relative risk of 2.85 for mortality in the ICU for those treated with acyclovir over the control group, implying that treatment may not have been advantageous in this cohort. Furthermore, this study did not identify creatinine levels as significant risk factors associated with ICU mortality in the acyclovir treated group. The multivariate analysis included a propensity score to adjust for potential treatment selection bias, yet the treated group still yielded a higher odds ratio for ICU mortality.

Bias cannot be excluded. On one hand, of the ten ICU-deceased patients that received acyclovir, six were placed on ECMO or ECCO2R. When ECMO patients are excluded from analysis, the difference in mortality between the acyclovir-treated and control group is no longer considered statistically significant (p = .120). A study by Hraiech et al., examining HSV-reactivation in severe ARDS patients with veno-venous ECMO, discovered no difference in clinical outcomes between treated and untreated patients apart from a trend toward longer duration of IMV for treated patients[30]. However, the authors of that study excluded all immunosuppressive patients defined by those who had received corticosteroids,

presented with neutropenia, or had undergone organ transplantation.

Secondary endpoints

Patients treated with acyclovir had a significantly longer length of ICU stay (41.8 vs. 26.8 days, p = .018). This finding is supported by a retrospective cohort study (n = 306) of Heimes et al.,5 wherein ICU and hospital LOS (31 vs. 24 days, p = .002 and 24 vs. 17 days, p < .001, respectively), as well as duration of IMV (18 vs. 11 days, p < .001), were significantly higher in acyclovir-treated patients.

The acyclovir group had a significantly lower P/F ratio (59.9 vs. 73.4 mmHg, p = .008), indicating that the treated group likely suffered from a more severe form of ARDS. However, only the lowest P/F ratio per patient was analyzed, making it a highly doubtful variable. A single deterioration during the entire ICU admission is sufficient to drastically alter the variable's value for that given patient. An alternate strategy could have been to either calculate mean P/F ratios per patient or to determine P/F ratios at specified moments during hospitalization, such as the P/F ratio at the time of HSV-1 discovery. Another relevant sub-analysis would be to categorize patients according to the Berlin classification into a mild (200 – 300 mmHg), moderate (100 – 200 mmHg), and severe (< 100 mmHg) form of ARDS[31].

Various biochemical markers regarding renal function and immune response were recorded. Although some trends can be assumed, such as lymphopenia in the acyclovir group (3.0 E9.l-1 vs. 4.9 E9.l-1, p = .058), the sample size of this study was too small to reach statistical significance in any of the laboratory findings. Moreover, a considerable amount of records had incomplete lab results, necessitating their exclusion from analysis and thereby reducing the cohort size of this sub-analysis even further.

Risk factors of HSV-1 reactivation

Airway HSV-1 reactivation occurred in 42 out of 121 ICU patients diagnosed with COVID-19 associated ARDS (34.7%). Literature reports of HSV-1 reactivation in this specific patient population vary widely, ranging from 5% to 83%,[9,14,16,32] likely reflecting diversified testing indications or the use of different samples for the presence of HSV-1 DNA. The only comorbidity associated with HSV-1 reactivation was chronic renal failure, with an odds ratio of 5.07 (95% CI 1.24 – 20.76, p = .031). This finding is substantiated by a large prospective community-based study (n = 9926) by Forbes et al.,[33] in which the authors discovered that 'ever diagnosis of kidney disease' was the sole comorbidity related with frequent HSV-1 reactivations in the general population (adjusted OR 1.87, 95% CI 1.02 – 3.40). In this study, ECMO was not statistically associated with HSV-1 reactivation (OR 1.80, 95% CI .73 – 4.47, p = .235).

Prolonged need for IMV appears to be a risk factor for HSV-1 reactivation with a mean duration of 29.8 days, compared to 22.2 days among HSV-1 negative patients, p = .008. According to another retrospective analysis (n = 18), HSV-1 reactivation occurred in the later disease phases, especially after longer periods of critical illness and mechanical ventilation.14 A prospective study by De Vos et al. (n = 105) found an association between the identification of HSV and extended IMV and ICU stay[3].

In addition, HSV-1 positive patients exhibited a higher maximal SOFA score (14.2 vs. 12.9, p = .015). With a SOFA score of 11 or above, both groups fall into the highest mortality risk category; hence the clinical significance of this discovery is uncertain[28]. Lastly, and more therapeutically relevant, HSV-1 positive patients required norepinephrine vasopressor support for a longer mean duration (15 vs. 10 days, p = .005).

Limitations

This study has several limitations, including a small sample size, a retrospective design, and potential confounding factors that were not accounted for in the analysis. Major determining factors were included as potential confounders in the matched propensity score analyses, but imbalances in unmeasured variables cannot be accounted for. Both a selection and information bias may have occurred by an underrepresented proportion of respiratory samples from non-invasively ventilated patients, resulting in a selection of patients with poorer prognosis and plausible underestimated incidence of herpes simplex reactivation, respectively. The critical care physician's decision on whether a patient received treatment with acyclovir may have also introduced selection bias. Moreover, as neither the sampling for HSV-detection nor the acyclovir treatment was protocolized, the initiation of the treatment relative to the start of the COVID-19 disease was highly variable. On the other hand, the treatment duration was always greater than five days (ranging from 5 up to 24 days) and the dosage was always 10 mg.kg-1 three times daily, unless adaptation to the renal function was required.

Future investigations

Single-center studies are known for their lack of generalizability, so multicenter studies or a meta-analysis from multiple smaller studies are warranted to be able to extrapolate these results. The rather small sample size limited the power to detect significant differences between the two groups,

further necessitating more extensive research. This study can therefore only be considered as preliminary: a follow-up study including all COVID-19 patients from our institution admitted to the ICU (n > 450) is being conceptualized during the writing of this article.

Conclusion

In this retrospective single-center cohort study, 34.7% of critically ill covid-19 patients had HSV-1 airway reactivation, of which 67% received treatment with acyclovir. Treated patients had a significantly higher mortality than controls, thereby adding to the contradictory literature regarding the role of acyclovir in patients with HSV-1 reactivation in the ICU. These results suggest that airway HSV-1 reactivation in critically ill patients with COVID-19 might be better left untreated. Although potential confounders were taken into account, treatment selection bias remains a plausible limiting factor, warranting larger prospective trials to either confirm or refute these findings.

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Authors' contributions: PJ, WV, TS, and NC conceptualized the design of the study. NC performed the database research, interpreted the analysis, and wrote the manuscript. WV, JK, and JJ carried out the data collection through database research. TS performed the statistical analysis and interpretation of the data. All authors read and approved the final manuscript.

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