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Epidemiology of Multiple Herpes Viremia in Previously Immunocompetent Patients With Septic Shock

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Background. Systemic reactivations of herpesviruses may occur in intensive care unit (ICU) patients, even in those without prior immune deficiency. However, the clinical relevance of these events is uncertain.

Methods. In this study we selected patients admitted with septic shock and treated for more than 4 days from a prospectively enrolled cohort of consecutive adults in the mixed ICUs of 2 tertiary care hospitals in the Netherlands. We excluded patients who had received antiviral treatment in the week before ICU admission and those with known immunodeficiency. We studied viremia episodes with cytomegalovirus (CMV), Epstein–Barr virus (EBV), human herpesvirus 6 (HHV-6), herpes simplex virus types 1 (HSV-1) and 2 (HSV-2), and varicella zoster virus (VZV) by weekly polymerase chain reaction in plasma.

Results. Among 329 patients, we observed 399 viremia episodes in 223 (68%) patients. Viremia with CMV, EBV, HHV-6, HSV-1, HSV-2, and VZV was detected in 60 (18%), 157 (48%), 80 (24%), 87 (26%), 13 (4%), and 2 (0.6%) patients, respectively; 112 (34%) patients had multiple concurrent viremia events. Crude mortality in the ICU was 36% in this latter group compared to 19% in remaining patients ($P < .01$). After adjustment for potential confounders, time-dependent bias, and competing risks, only concurrent CMV and EBV reactivations remained independently associated with increased mortality (adjusted subdistribution hazard ratio, 3.17; 95% confidence interval, 1.41–7.13).

Conclusions. Herpesvirus reactivations were documented in 68% of septic shock patients without prior immunodeficiency and frequently occurred simultaneously. Concurrent reactivations could be independently associated with mortality.

Clinical Trials Registration. NCT01905033.

Keywords. cytomegalovirus; Epstein–Barr virus; human herpesvirus 6; reactivation; mortality.

Herpesviruses remain dormant in various types of human cells after primary infection and thus may reactivate at a later time when the host is in an immunosuppressed state. In fact, systemic reactivation of latent herpesviruses is assumed to be the main mechanism responsible for the presence of viral DNA in blood [1]. However, viral reactivation is not exclusive to "classic" high-risk groups, such as patients infected with the human immunodeficiency virus (HIV), patients receiving chemotherapy, and transplant recipients and may also occur in previously immunocompetent patients when they suffer from severe and prolonged critical illness. Indeed, systemic reactivations of herpesviruses have frequently been reported in intensive care unit (ICU) patients and in those without known prior immune deficiency [2–4].

Most clinical studies in critically ill patients have primarily focused on cytomegalovirus (CMV) infections, for which most data supporting an independent association with increased mortality have been documented [3, 5–9]. However, viremia by other types of herpesviruses, such as Epstein–Barr virus (EBV), human herpesvirus 6 (HHV-6), and herpes simplex virus (HSV), has also been described [3, 10–12]. Nevertheless, it remains controversial whether such reactivations constitute a true cause or are merely a marker of increased morbidity and mortality in critically ill patients, because most studies were hampered by a limited sample size and used insufficient methodologies to accurately assess outcome relations while taking into account different kinds of bias.

Our aims in this study were to describe the incidences of CMV, EBV, HHV-6, HSV type 1 (HSV-1), HSV type 2 (HSV-2), and varicella zoster virus (VZV) viremia in unselected patients admitted to the ICU with septic shock and to assess their associations with mortality.

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METHODS

Patients and Measurements

This study was conducted within the framework of the Molecular Diagnosis and Risk Stratification of Sepsis (MARS) cohort for which the institutional review board approved an opt-out method of informed consent (protocol number 10-056C) [13]. We prospectively enrolled consecutive adults who presented with septic shock to the mixed ICUs of 2 tertiary care centers in the Netherlands between January 2011 and June 2014 and remained in the ICU beyond day 4. Septic shock was defined by the presence of sepsis plus the use of noradrenaline for hypotension in a dose of >0.1 μg/kg/min for more than 12 hours during the first 3 days in the ICU, implying that included patients met both the 2003 and 2016 consensus criteria for septic shock [14, 15]. All clinically diagnosed infections were confirmed by a post hoc physician review according to the Centers for Disease Control and Prevention International Sepsis Forum Consensus Conference definitions that were translated and adapted to the Dutch situation [13]. We excluded patients who had received (val)ganciclovir, (val) aciclovir, cidofovir, or foscarnet in the week before ICU admission and those with known immunodeficiency as defined by a history of solid organ or stem cell transplantation, infection with HIV, hematological malignancy, use of immunosuppressive medication (prednisone >0.1 mg/kg for >3 months, prednisone >75 mg/day for >1 week, or equivalent), chemotherapy, or radiotherapy in the year before ICU admission, and any known humoral or cellular immune deficiency.

Leftover plasma obtained daily as part of routine patient care was stored within 4 hours after blood draw at −80°C until further processing. CMV and HSV serostatus were determined in plasma samples at admission using enzyme immunoassays (Enzygnost, Siemens Healthcare Diagnostic Products, Marburg, Germany). We did not determine the EBV, HHV-6, and VZV serostatus because the seroprevalence of these viruses is known to be very high (ranging from 90% to 100%) in adult populations [16–18]. Subsequently, viral loads of CMV, EBV, and HHV-6 in plasma were determined weekly, starting from ICU admission until ICU discharge, using quantitative real-time TaqMan polymerase chain reaction (PCR) [19–21]. For intermediary days on which PCR was not performed, we estimated viral loads using log-linear imputation.

CMV and EBV viremia was defined by a viral load >100 IU/mL as calibrated according to the World Health Organization Standards. HHV-6 viremia was defined by a viral load >100 copies/mL, calibrated to an electron microscopically defined standard [21]. Of note, the limit of quantification for the HHV-6 assay was 1000 copies/mL. Below this limit viremia events could be detected, but quantification was unreliable. HSV-1, HSV-2, and VZV were measured using real-time TaqMan PCR, yielding only qualitative results. Screening for herpes virus reactivations was not part of routine clinical practice. Neither serology results nor viral loads measured during our study were made available to the treating physicians.

Data Analyses

The incidences of viremia and their associations with patient characteristics were compared using χ^2 test and nonparametric descriptive statistics (ie, Wilcoxon rank-sum test). We used survival regression analyses to assess the association between viremia and clinical outcome. In these analyses, ICU discharge and ICU death were considered competing events because in case mortality is the event of interest, discharge alive from the ICU precludes this event of interest from being observed [22]. Furthermore, patients who are discharged at a certain time point are in a better health state than those who remain in the ICU beyond that same time point and remain at risk for death [23, 24]. Therefore, competing risks Cox proportional hazard regressions were used to fit multivariable models with viremia status as a time-dependent exposure variable. A competing risks analysis provides 2 measures of association. First, the cause-specific hazard ratio describes the instantaneous effect on the outcome of interest, given that the patient is still at risk. In our case, it estimates the direct effect of viremia on the rates of death and discharge in the ICU. Second, the subdistribution hazard ratio (SHR) for mortality describes the cumulative risk of dying from viremia while accounting for the competing risk of ICU discharge.

In multivariable analyses we accounted for (possible) confounding by adjusting for Acute Physiology and Chronic Health Evaluation (APACHE) IV score as a summary measure of disease severity upon ICU admission, prior ICU admission during the hospital stay, surgery in the week before ICU admission, and Charlson comorbidity index. To estimate the population-attributable fraction of mortality due to each type of viremia (ie, the fraction of ICU mortality that is attributable to viremia on the population level), we used multistate models that take the time-dependent nature of viremia occurrence and competing events into account (Supplementary Figure 1) [25]. Confidence intervals (CIs) for effect estimates were calculated using bootstrap resampling [26, 27].

Despite our efforts to accurately assess the effect of each viremia on outcome, residual confounding may still remain because markers of illness at baseline may no longer be representative of the disease state at the time of viremia onset. Therefore, in the final analyses, focusing on the most prevalent combinations of viremia, we used marginal structural models to also adjust for the evolution of disease severity prior to the onset of viremia [28–30]. To this end, these models included the following 2 time-varying covariables: the sequential organ failure assessment score as a daily marker of illness severity and use of high-dosed corticosteroids as a daily marker of

immunosuppressive therapy in the ICU. Marginal structural model analysis first involves estimation of the daily probabilities of viremia onset using a multivariable logistic regression model that includes patient and disease characteristics measured on a daily basis. Subsequently, these probabilities are used to calculate inverse probability weights, which are then included as covariables in a final Cox regression model. Data were analyzed using SAS 9.2 (Cary, North Carolina) and R 2.15.1 software (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

We screened 437 patients with septic shock and with an ICU admission longer than 4 days. Among these, 108 were not eligible for study inclusion because of known prior immune deficiency or previous antiviral treatment, yielding 329 patients for study inclusion. Most septic shock events at ICU admission were attributed to bacterial pathogens including *Enterococcus* species (17%), *Escherichia coli* (15%), *Staphylococcus aureus* (10%), *Pseudomonas aeruginosa* (7%), and *Enterobacter* species (7%) (Supplementary Table 1). In 25% of patients the causative pathogen remained unknown.

Incidence of Viral Reactivations

During 862 weekly observations, 399 episodes of viremia were detected in 223 (68%) patients; 112 (34%) developed multiple viremia events (ie, with different viruses) concurrently. The proportion of viremia did not differ between individuals presenting to the ICU for the first time and those who had been admitted previously (67% vs 69%, $P = .84$). Patients who developed viral reactivation had higher lactate and C-reactive protein levels upon ICU admission, and more frequently had an abdominal focus as the source of their infection (34% vs 18%, $P = .01$) compared to patients without (ever) viremia (Table 1, Supplementary Table 2).

Cumulative incidences of viral reactivation were 27% among 214 (65%) CMV seropositive patients and 31% among 277 (84%) HSV-1 seropositive patients. In addition, we observed 2 cases of CMV viremia and 1 case of HSV-1 viremia among patients with a seronegative status at the time of ICU admission. EBV viremia occurred in 157 (48%) and HHV-6 viremia occurred in 80 (24%) patients, whereas VZV was detected in only 2 patients (0.6%).

Point Prevalence of Viremia at Admission and on Days 7, 14, 21, and 28 in the ICU

The prevalence of (any) viremia event at ICU admission was 36%, and this occurrence increased to 86% in patients who remained in the ICU for 4 weeks or longer (Figure 1). Among patients who had survived until day 28 in the ICU, 33% had viral loads of EBV >1000 IU/mL and 17% had viral loads of CMV >1000 IU/mL (Figure 2). In total, 189 of 223 (85%) patients with viremia remained virus positive at least until ICU discharge; this proportion was higher for CMV (92%) than for EBV (68%), HSV-1 (53%), or HHV-6 (39%).

Table 1. Baseline Characteristics of Septic Shock Patients by Viremia Status

Patient Characteristic	Ever Viremia (n = 223)	Never Viremia (n = 106)	*P* Value
Age (y)	65 (57–74)	66 (54–73)	.36
Male gender	146 (65)	62 (58)	.22
Non-European descent	28 (13)	11 (10)	.57
Body mass index (kg/m^2)	25 (22–28)	25 (22–29)	.60
Prior ICU admission	57 (26)	26 (25)	.84
Medical admission	153 (69)	71 (67)	.77
Charlson comorbidity index[a]	4.6 (0–11)	4.6 (0.0–10.6)	.73
Acute Physiology and Chronic Health Evaluation (APACHE) IV score[b]	85 (10–109)	82 (69–99)	.16
Plasma lactate[c]	3.8 (2.3–7.0)	2.8 (1.8–4.5)	<.01
C-reactive protein[d]	168 (83–280)	85 (27–207)	<.01
Source of infection			.01
Pulmonary	95 (43)	55 (52)	
Abdominal	76 (34)	19 (18)	
Other	52 (23)	32 (30)	
High-dose corticosteroid use in ICU[e]	129 (58)	40 (38)	<.01

Data are presented as medians (interquartile range) or absolute numbers (%). *P* values were calculated using nonparametric and χ^2 tests, respectively.

Abbreviation: ICU, intensive care unit.

[a]The Charlson comorbidity index reflects chronic comorbidities present prior to the current hospital admission.

[b]The APACHE IV score was calculated based on observations and measurements performed during the first 24 hours of ICU admission.

[c]Highest plasma lactate in first 24 hours of ICU admission. Of note, lactate levels were missing in 26 (8%) patients.

[d]First measured C-reactive protein in the first 24 hours of ICU admission.

[e]High-dose corticosteroid was defined as a daily dose of ≥250 mg hydrocortisone or equivalent during the first 4 days in the ICU.

Associations With Clinical Outcome

Crude ICU mortality was highest in patients with CMV (33%) and HHV-6 (33%) viremia, followed by EBV (31%), HSV-1 (29%), and HSV-2 (15%) viremia. After adjustment for potential confounders and taking into account competing risks and the time-dependent nature of virus reactivation, we found that no single viremia event remained significantly associated with ICU mortality by itself (Table 2). The population-attributable fractions of ICU mortality, which is dependent on both the incidence and the virulence of the virus, were EBV (25%; 95% CI, 5%–44%), HHV-6 (18%; 95% CI, 4%–42%), and CMV (12%; 95% CI, 0%–24%). Of note, the CIs for these estimates showed large overlap.

Patients with multiple viremia events during their ICU stay had higher ICU mortality (36%) compared to those with either a single-type viremia (15%) or no viremia (23%; $P < .01$). The mortality was highest in the group of patients who had both CMV and EBV viremia (51%), followed by those with combined

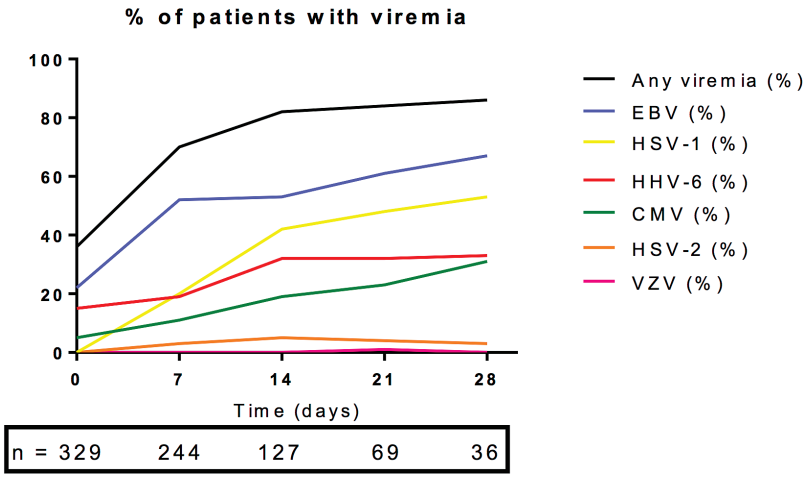

Figure 1. Proportion of patients with viremia. Proportions were calculated at intensive care unit (ICU) admission and on days 7, 14, 21, and 28 among patients who remained in the ICU beyond that day. The total number of patients at each time point is shown along the bottom. Day 1 corresponds to the first 24 hours in the ICU. Cytomegalovirus and Epstein–Barr virus viremia were defined as a viral load >100 IU/mL. Human herpesvirus 6 viremia was defined as a viral load >100 copies/mL. Herpes simplex virus and varicella zoster virus were qualitative measurements (yes/no viremia). Abbreviations: CMV, cytomegalovirus; EBV, Epstein–Barr virus; HSV-1, herpes simplex virus types 1; HSV-2, herpes simplex virus types 2; HHV-6, human herpesvirus 6; VZV, varicella zoster virus

CMV and HHV-6 viremia (43%) and with EBV and HHV-6 viremia (41%).

Subsequently, we further explored the combined occurrences of CMV, EBV, and HHV-6 viremia because these viruses were associated with the highest crude mortalities, highest adjusted hazard ratios for mortality, and highest population-attributable fractions of mortality (Table 2). For each combination of viremia, a new variable was created that consisted of 2 levels, of which 1 level was combined viremia and the other level was the remaining, for example, (a) both CMV and EBV viremia and (b) only CMV viremia, only EBV viremia, or no viremia. Concurrent CMV and EBV viremia remained significantly associated with increased mortality in multivariable analyses using marginal structural modeling (SHR, 3.17; 95% CI, 1.41–7.13; Table 3).

Sensitivity Analyses

To test the robustness of our findings, we performed sensitivity analyses using higher cutoff values to define the onset of CMV and EBV viremia. Patients with EBV loads greater than 500 and 1000 IU/mL had higher death rates than those with no (or only low level) EBV reactivation (adjusted SHR, 1.86; 95% CI, 1.02–3.40 and adjusted SHR, 2.09; 95% CI, 1.08–4.05, respectively). In contrast, higher CMV loads of >500 or >1000 IU/mL were not significantly associated with increased mortality (adjusted SHR, 1.54; 95% CI, 0.56–4.25 and adjusted SHR, 2.52; 95% CI 0.75–8.43, respectively).

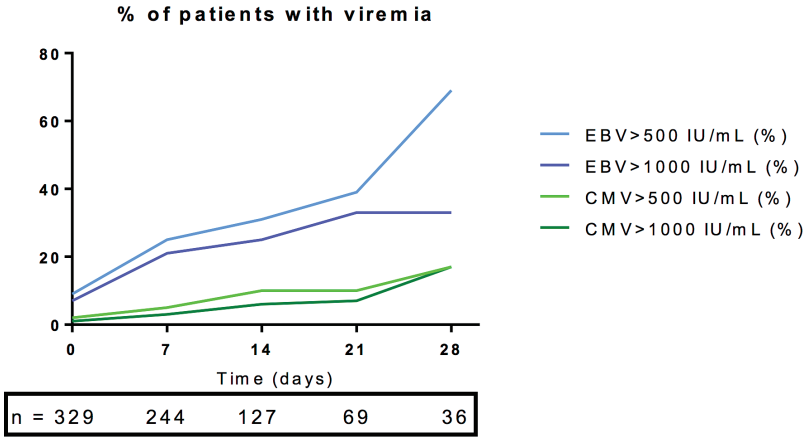

Figure 2. Occurrences of cytomegalovirus (CMV) and Epstein–Barr virus (EBV) viremia above 500 and 1000 IU/mL. Proportions were calculated at intensive care unit (ICU) admission and on days 7, 14, 21, and 28 among patients who remained in the ICU beyond that day. Day 1 corresponds to the first 24 hours in the ICU. Only viral loads of CMV, EBV, and human herpesvirus 6 (HHV-6) were quantitatively determined. However, quantification of HHV-6 viral load was unreliable below 1000 copies/mL. Therefore, in this figure, only CMV and EBV are presented.

Table 2. Associations Between Viremia and Clinical Outcome

Cox Model (n = 329)	Cumulative Incidence of Viremia	ICU Discharge (CSHR)	Death in ICU (CSHR)	Death in ICU (SHR)	Population-Attributable Fraction of ICU Mortality at Day 30
Individual viremia					
Cytomegalovirus	60 (18)	1.07 (0.77–1.49)	1.61 (0.94–2.75)	1.62 (0.80–3.25)	0.12 (0.00–0.24)
Epstein–Barr virus	157 (48)	0.75 (0.57–0.98)	1.18 (0.70–2.01)	1.61 (0.88–2.92)	0.25 (0.05–0.44)
Human herpesvirus 6	80 (24)	0.80 (0.59–1.10)	1.20 (0.71–2.03)	1.41 (0.74–2.68)	0.18 (0.04–0.42)
Herpes simplex virus type 1	87 (26)	0.84 (0.63–1.12)	1.22 (0.73–2.05)	1.27 (0.65–2.49)	0.09 (-0.05–0.22)
Herpes simplex virus type 2	13 (4)	NA	NA	NA	NA
Varicella zoster virus	2 (1)	NA	NA	NA	NA
Multiple viremia					
≥2 concurrent viremia events	112 (34)	0.79 (0.59–1.06)	1.52 (0.91–2.52)	1.90 (0.98–3.69)	0.23 (0.07–0.40)

Abbreviations: CSHR, cause-specific hazard ratio; ICU, intensive care unit; NA, not applicable, no multivariable analyses because of low number of events; SHR, Subdistribution Hazard Ratio.

Data are presented as absolute numbers (%) or hazard ratios with 95% confidence intervals. The hazard ratios are derived from multivariable models with adjustment for the time-varying onset of viremia and baseline differences at ICU admission, including Acute Physiology and Chronic Health Evaluation (APACHE) IV score, prior ICU admission during the hospital stay, surgical reason for admission, and Charlson comorbidity index.

CSHR estimates the direct effect of viremia on clinical outcome (ie, ICU discharge or death). The subdistribution hazard ratio is a summary measure of both separate cause-specific hazards and estimates the overall risk of dying from viremia while taking into account the competing event of discharge alive from the ICU.

DISCUSSION

In this cohort of previously immunocompetent patients presenting with septic shock, a 68% majority developed herpes viremia while in the ICU. One-third of patients had multiple concurrent viremia episodes, with dual CMV and EBV viremia being independently associated with mortality.

Previous studies have demonstrated independent associations between CMV reactivation and poor outcome [3, 5–9],

Table 3. Associations Between Concurrent Viremia Events and Clinical Outcome

Cox Model	ICU Discharge (CSHR)	Death in ICU (CSHR)	Death in ICU (SHR)
Concurrent cytomegalovirus and Epstein–Barr virus viremia			
Crude model with adjustment for			
time-varying onset of viremia	0.69 (0.44–1.08)	2.54 (1.43–4.50)	3.48 (1.60–7.58)
Multivariable model with adjustment for			
time-varying onset of viremia			
severity of illness at ICU admission[a]			
evolution of disease prior to viremia[b]	0.83 (0.54–1.27)	2.44 (1.33–4.49)	3.17 (1.41–7.13)
Concurrent cytomegalovirus and human herpesvirus 6 viremia			
Crude model with adjustment for			
time-varying onset of viremia	1.14 (0.72–1.79)	2.52 (1.24–5.11)	2.67 (0.95–7.49)
Multivariable model with adjustment for			
time-varying onset of viremia			
severity of illness at ICU admission[a]			
evolution of disease prior to viremia[b]	1.15 (0.71–1.87)	2.39 (1.15–4.98)	2.51 (0.88–7.18)
Concurrent Epstein–Barr virus and human herpesvirus 6 viremia			
Crude model with adjustment for			
time-varying onset of viremia	0.78 (0.54–1.12)	1.81 (1.06–3.08)	2.16 (1.11–4.23)
Multivariable model with adjustment for			
time-varying onset of viremia			
severity of illness at ICU admission[a]			
evolution of disease prior to viremia[b]	0.87 (0.61–1.24)	1.55 (0.87–2.77)	1.87 (0.91–3.86)

Abbreviations: CSHR, cause-specific hazard ratio; ICU, intensive care unit; SHR, Subdistribution Hazard Ratio.

In each of the 3 different Cox regression models, all 329 patients were included, but in every model a different combination of viremia was assessed as follows: combined cytomegalovirus (CMV) and Epstein–Barr virus (EBV) viremia (n = 38), CMV and human herpesvirus 6 (HHV-6) (n = 22), or EBV and HHV-6 (n = 54), respectively.

Data are presented as hazard ratios with 95% confidence intervals. CSHR estimates the direct effect of viremia on clinical outcome (ie, ICU discharge or death). The subdistribution hazard ratio is a summary measure of both separate cause-specific hazards and estimates the overall risk of dying from viremia while taking into account the competing event of discharge alive from the ICU.

[a]Adjusted for Acute Physiology and Chronic Health Evaluation (APACHE) IV score, prior ICU admission during the hospital stay, surgical reason for admission, and Charlson comorbidity index

[b]Adjusted for time-dependent covariables, including sequential organ failure assessment score (using a 48-hour time lag to avoid statistical overcorrection) and use of high-dose corticosteroid therapy (defined by a daily hydrocortisone dose of ≥250 mg or equivalent).

but most have largely neglected the potential etiologic role of concurrent (other) viral reactivations. Perhaps the immunomodulatory effects of CMV that have been shown to decrease the host defenses against bacterial and fungal infections [31, 32] could also hold for other herpesviruses. More specifically, our study suggests that any potential impact of CMV on outcome may be modified by the concurrent reactivation of EBV in particular, as CMV reactivation by itself was not associated with mortality in septic shock patients.

Our findings of frequently occurring multiple viremia in ICU patients confirms the results of a previous study in which cumulative incidences of viremia that ranged from 10% to 53% during the first 30 days in the ICU were reported [3]. Important differences between both studies relate to the selected patient population and the method of viremia detection. In our study we exclusively investigated severely ill patients with septic shock as compared to a more heterogeneous group that included sepsis patients without organ dysfunction in the previous study. In addition, that study indiscriminately tested whole blood or plasma for the presence of viral DNA as compared to only plasma used in our study. Inclusion of whole blood samples may have resulted in the detection of nonreplicating (latent) viruses, which probably bears little clinical consequence. Furthermore, we used higher cutoff levels in our study to define relevant viremia events in order to better distinguish true viremia from test positivity due to viral DNA that is redistributed from lytic cells to plasma, as has been observed for CMV [33].

Although EBV viremia has been associated with increased morbidity and mortality in critically ill patients with prolonged lengths of stay in the ICU [10], these findings were neither confirmed in the previous study by Walton et al [3] nor in our own main analysis. EBV interacts with CD8+ and CD4+ T-cell surveillance [34], which may have implications for the susceptibility of the host to other infections, including CMV reactivation. However, evidence for this is still lacking, and it is possible that EBV represents no more than a generic marker of a suppressed immune system. Further studies should focus on possible underlying mechanisms by which combined CMV and EBV reactivation may contribute to morbidity and mortality in these patients.

Our study has several strengths. First, our study differs from previous studies in that we assessed the associations between virus reactivations and outcome while accounting for different kinds of bias, including adjustment for the time-dependent nature of exposure and the evolution of disease severity prior to viremia onset [3]. Second, our study was nested within a large prospective data collection initiative that included consecutive patients, thereby minimizing selection bias [13]. In contrast, the most important study limitation relates to its observational design, which precludes making strong inferences about possible causal associations between viremia events and outcome.

Currently, critically ill patients are not routinely screened for systemic herpesvirus reactivation during their stay in the ICU. This is in contrast to the prophylactic and preemptive strategies that are generally used in transplant recipients or patients with hematological malignancies. Although experimental studies are needed to elucidate the pathophysiology of herpesvirus reactivation and its consequences for the host, large observational studies such as ours remain necessary to identify patients who are both at risk for and vulnerable to the effects of virus reactivation. Sepsis and the acute respiratory distress syndrome are well-known risk factors for herpesvirus reactivation [2–4, 9]. Although we did not develop a formal prediction model, both an abdominal source of infection and high-dose corticosteroid use also appear to be possible risk factors for viremia based on our data (Table 1).

The success of any antiviral treatment strategy in ICU patients will largely depend on the ability to predict which patients may benefit most from such interventions. It remains to be investigated whether antiviral treatment strategies, especially directed against CMV, are effective in reducing morbidity and mortality in critically ill patients. Intervention trials comparing prophylaxis, preemptive treatment, and wait-and-see strategies are necessary before any evidence-based recommendations regarding the clinical management of herpesvirus reactivation in ICU patients can be given.

In conclusion, critically ill patients with septic shock are prone to develop reactivations due to various types of herpesviruses while in the ICU, even if they were previously immunocompetent. More importantly, we observed increased mortality rates for patients having multiple concurrent viremia events, most markedly in patients with combined CMV and EBV reactivation. Using multivariable analyses to account for competing events, time-varying onset of viremia, baseline differences between patients, and the evolution of disease severity until the onset of viral reactivation, our data suggest that multiple herpesvirus reactivations are independently associated with mortality. Future studies are needed to confirm these findings.

Supplementary data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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References

1. Limaye AP, Boeckh M. CMV in critically ill patients: pathogen or bystander? Rev Med Virol **2010**; 20:372–9.
2. Osawa R, Singh N. Cytomegalovirus infection in critically ill patients: a systematic review. Crit Care **2009**; 13:R68.
3. Walton AH, Muenzer JT, Rasche D, et al. Reactivation of multiple viruses in patients with sepsis. PLoS One **2014**; 9:e98819.
4. Ong DS, Spitoni C, Klein Klouwenberg PM, et al. Cytomegalovirus reactivation and mortality in patients with acute respiratory distress syndrome. Intensive Care Med **2016**; 42:333–41.
5. Chiche L, Forel JM, Roch A, et al. Active cytomegalovirus infection is common in mechanically ventilated medical intensive care unit patients. Crit Care Med **2009**; 37:1850–7.
6. Coisel Y, Bousbia S, Forel JM, et al. Cytomegalovirus and herpes simplex virus effect on the prognosis of mechanically ventilated patients suspected to have ventilator-associated pneumonia. PLoS One **2012**; 7:e51340.
7. Limaye AP, Kirby KA, Rubenfeld GD, et al. Cytomegalovirus reactivation in critically ill immunocompetent patients. JAMA **2008**; 300:413–22.
8. Ziemann M, Sedemund-Adib B, Reiland P, Schmucker P, Hennig H. Increased mortality in long-term intensive care patients with active cytomegalovirus infection. Crit Care Med **2008**; 36:3145–50.
9. Ong DSY, Klein Klouwenberg PMC, Verduyn Lunel FM, et al. Cytomegalovirus seroprevalence as a risk factor for poor outcome in acute respiratory distress syndrome*. Crit Care Med **2015**; 43:394–400.
10. Libert N, Bigaillon C, Chargari C, et al. Epstein-Barr virus reactivation in critically ill immunocompetent patients. Biomed J **2015**; 38:70–6.
11. Razonable RR, Fanning C, Brown RA, et al. Selective reactivation of human herpesvirus 6 variant a occurs in critically ill immunocompetent hosts. J Infect Dis **2002**; 185:110–3.
12. Lepiller Q, Sueur C, Solis M, et al. Clinical relevance of herpes simplex virus viremia in intensive care unit patients. J Infect **2015**; 71:93–100.
13. Klein Klouwenberg PMC, Ong DSY, Bos LDJ, et al. Interobserver agreement of Centers for Disease Control and Prevention criteria for classifying infections in critically ill patients. Crit Care Med **2013**; 41:2373–8.
14. Levy MM, Fink MP, Marshall JC, et al. 2001 SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference. Springer-Verlag: **2003**:530–8.
15. Singer M, Deutschman CS, Seymour CW, et al. The third international consensus definitions for sepsis and septic shock (Sepsis-3). JAMA **2016**; 315:801–10.
16. De Paschale M, Clerici P. Serological diagnosis of Epstein-Barr virus infection: problems and solutions. World J Virol **2012**; 1:31–43.
17. Zerr DM, Meier AS, Selke SS, et al. A population-based study of primary human herpesvirus 6 infection. N Engl J Med **2005**; 352:768–76.
18. Nardone A, de Ory F, Carton M, et al. The comparative sero-epidemiology of varicella zoster virus in 11 countries in the European region. Vaccine **2007**; 25:7866–72.
19. van Doornum GJ, Guldemeester J, Osterhaus AD, Niesters HG. Diagnosing herpesvirus infections by real-time amplification and rapid culture. J Clin Microbiol **2003**; 41:576–80.
20. Niesters HG, van Esser J, Fries E, Wolthers KC, Cornelissen J, Osterhaus AD. Development of a real-time quantitative assay for detection of Epstein-Barr virus. J Clin Microbiol **2000**; 38:712–5.
21. de Pagter PJ, Schuurman R, Visscher H, et al. Human herpes virus 6 plasma DNA positivity after hematopoietic stem cell transplantation in children: an important risk factor for clinical outcome. Biol Blood Marrow Transplant **2008**; 14:831–9.
22. Wolkewitz M, Cooper BS, Bonten MJ, Barnett AG, Schumacher M. Interpreting and comparing risks in the presence of competing events. BMJ **2014**; 349:g5060.
23. Bekaert M, Vansteelandt S, Mertens K. Adjusting for time-varying confounding in the subdistribution analysis of a competing risk. Lifetime Data Anal **2010**; 16:45–70.
24. Wolkewitz M, Beyersmann J, Gastmeier P, Schumacher M. Modeling the effect of time-dependent exposure on intensive care unit mortality. Intensive Care Med **2009**; 35:826–32.
25. Schumacher M, Wangler M, Wolkewitz M, Beyersmann J. Attributable mortality due to nosocomial infections. A simple and useful application of multistate models. Methods Inf Med **2007**; 46:595–600.
26. Beyersmann J, Gastmeier P, Grundmann H, et al. Use of multistate models to assess prolongation of intensive care unit stay due to nosocomial infection. Infect Control Hosp Epidemiol **2006**; 27:493–9.
27. Barnett AG, Beyersmann J, Allignol A, Rosenthal VD, Graves N, Wolkewitz M. The time-dependent bias and its effect on extra length of stay due to nosocomial infection. Value Health **2011**; 14:381–6.
28. Robins JM, Hernán MA, Brumback B. Marginal structural models and causal inference in epidemiology. Epidemiology **2000**; 11:550–60.
29. Bekaert M, Timsit JF, Vansteelandt S, et al; Outcomerea Study Group. Attributable mortality of ventilator-associated pneumonia: a reappraisal using causal analysis. Am J Respir Crit Care Med **2011**; 184:1133–9.
30. Ong DSY, Bonten MJM, Safdari K, et al. Epidemiology, management and risk-adjusted mortality of ICU-acquired enterococcal bacteremia. Clin Infect Dis **2015**; 61:1413–20.
31. Papazian L, Hraiech S, Lehingue S, et al. Cytomegalovirus reactivation in ICU patients. Intensive Care Med **2016**; 42:28–37.
32. Nichols WG, Corey L, Gooley T, Davis C, Boeckh M. High risk of death due to bacterial and fungal infection among cytomegalovirus (CMV)-seronegative recipients of stem cell transplants from seropositive donors: evidence for indirect effects of primary CMV infection. J Infect Dis **2002**; 185:273–82.
33. Schäfer P, Tenschert W, Schröter M, Gutensohn K, Laufs R. False-positive results of plasma PCR for cytomegalovirus DNA due to delayed sample preparation. J Clin Microbiol **2000**; 38:3249–53.
34. Ressing ME, Horst D, Griffin BD, et al. Epstein-Barr virus evasion of CD8(+) and CD4(+) T cell immunity via concerted actions of multiple gene products. Semin Cancer Biol **2008**; 18:397–408.