PRELIMINARY COMMUNICATION

Cytomegalovirus Reactivation in Critically Ill Immunocompetent Patients

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Context Cytomegalovirus (CMV) infection is associated with adverse clinical outcomes in immunosuppressed persons, but the incidence and association of CMV reactivation with adverse outcomes in critically ill persons lacking evidence of immunosuppression have not been well defined.

Objective To determine the association of CMV reactivation with intensive care unit (ICU) and hospital length of stay in critically ill immunocompetent persons.

Design, Setting, and Participants We prospectively assessed CMV plasma DNAemia by thrice-weekly real-time polymerase chain reaction (PCR) and clinical outcomes in a cohort of 120 CMV-seropositive, immunocompetent adults admitted to 1 of 6 ICUs at 2 separate hospitals at a large US tertiary care academic medical center between 2004 and 2006. Clinical measurements were assessed by personnel blinded to CMV PCR results. Risk factors for CMV reactivation and association with hospital and ICU length of stay were assessed by multivariable logistic regression and proportional odds models.

Main Outcome Measures Association of CMV reactivation with prolonged hospital length of stay or death.

Results The primary composite end point of continued hospitalization (n=35) or death (n=10) by 30 days occurred in 45 (35%) of the 120 patients. Cytomegalovirus viremia at any level occurred in 33% (39/120; 95% confidence interval [CI], 24%-41%) at a median of 12 days (range, 3-57 days) and CMV viremia greater than 1000 copies/mL occurred in 20% (24/120; 95% CI, 13%-28%) at a median of 26 days (range, 9-56 days). By logistic regression, CMV infection at any level (adjusted odds ratio [OR], 4.3; 95% CI, 1.6-11.9; $P=.005$) and at greater than 1000 copies/mL (adjusted OR, 13.9; 95% CI, 3.2-60; $P<.001$) and the average CMV area under the curve (AUC) in $\log_{10}$ copies per milliliter (adjusted OR, 2.1; 95% CI, 1.3-3.2; $P<.001$) were independently associated with hospitalization or death by 30 days. In multivariable partial proportional odds models, both CMV 7-day moving average (OR, 5.1; 95% CI, 2.9-9.1; $P<.001$) and CMV AUC (OR, 3.2; 95% CI, 2.1-4.7; $P<.001$) were independently associated with a hospital length of stay of at least 14 days.

Conclusions These preliminary findings suggest that reactivation of CMV occurs frequently in critically ill immunocompetent patients and is associated with prolonged hospitalization or death. A controlled trial of CMV prophylaxis in this setting is warranted.

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CYTOMEGALOVIRUS (CMV) HAS long been recognized as an important viral pathogen in immunocompromised hosts. In addition to direct effects of CMV due to viral replication and resultant tissue injury, a range of indirect effects have been attributed to CMV in immunocompromised patients, including increased risk of secondary bacterial and fungal infections,[1-5] predisposition to specific malignancies such as Epstein-Barr virus–associated posttransplant lymphoproliferative disorder,[6] cardiovascular disease,[7,8] and mortality.[2-5,9,10] A causal role of CMV in mediating these indirect effects is supported by studies of antiviral prophylaxis in immunosuppressed patients demonstrating reductions in secondary bacterial and fungal infections,[2-5] hospitalization,[11] and mortality.[2-5]

The role of CMV infection in immunocompetent patients with critical illness has been investigated in several prior studies.[12-20] Although these studies used various virologic and statistical methods and designs, most demonstrated that CMV infection occurs commonly in critically ill patients and is associated with 1 or more adverse clinical outcomes.[12-20] However, these prior studies had 1 or more significant limitations, including relatively small sample size, inclusion of only selected types of intensive care unit (ICU) patients, lack of quantitative methods for

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CMV detection, nonblinded assessment of clinical end points, and/or failure to include comprehensive and rigorous statistical analyses. To address some of these limitations, we prospectively assessed CMV plasma DNAemia by real-time polymerase chain reaction (PCR) and clinical outcomes in a broad cohort of consecutive CMV-seropositive, immunocompetent adults admitted to an ICU, with the goal of defining the incidence, risk factors, timing, and association of CMV reactivation with clinically significant outcomes.

METHODS

Study Design

This prospective study was conducted at 6 ICUs at 2 separate hospitals at a large university-affiliated academic medical center between 2004 and 2006. The study was approved by the human subjects division at the University of Washington and written informed consent was obtained from study participants. Daily screening of new medical-surgical admissions to each ICU (burn [BICU], cardiac care [CICU], medical [MICU], and trauma [TICU]) was performed by study personnel, and patients who met other inclusion criteria underwent CMV serologic screening within 24 hours. All patients meeting study inclusion criteria were offered participation regardless of racial/ethnic status. Participants' racial/ethnic status was recorded as listed in the admitting/registration information and was collected in compliance with reporting requirements for National Institutes of Health–funded clinical studies.

Only patients who were newly admitted to the ICU from home or a baseline residential setting were included (ie, patients who were transferred to the ICU from within the hospital were excluded). Those who were CMV-seronegative were excluded from further study. Cytomegalovirus-seropositive patients who met all other inclusion criteria were enrolled and underwent prospective clinical assessments using standardized data collection forms. In addition, plasma samples were collected 3 times weekly and stored at −20°C for subsequent CMV PCR analysis. All clinical information was collected prospectively by study personnel who were blinded to the CMV PCR results (which were performed after all clinical data had been compiled). Patients were followed up prospectively until death or hospital discharge. Deaths occurring within 90 days after discharge from the hospital were assessed using state and national death registry data.

Inclusion and Exclusion Criteria

The inclusion criteria included ability to give informed consent (either patient or next of kin); age at least 18 years; admission to the BICU with at least 40% body surface burn or at least 20% body surface burn with inhalation injury, to the TICU with an Injury Severity Score higher than 15 and more than 4 U packed red blood cells within 24 hours, to the MICU with suspected or documented sepsis, or to the CICU with a diagnosis of acute myocardial infarction; expected survival more than 72 hours; and CMV seropositivity. The exclusion criteria were inability to give informed consent; age younger than 18 years; expected survival less than 72 hours; use of the antiviral agents cidofovir, foscarnet, ganciclovir, or valacyclovir (herpes simplex virus treatment doses of acyclovir, valacyclovir, or famciclovir were permitted) within the last 7 days; known or suspected human immunodeficiency virus infection; and known or suspected underlying immune deficiency (transplant, congenital immunodeficiency, or receipt of immunosuppressive medications [prednisone, azathioprine, tacrolimus, cyclosporin, sirolimus, or cyclophosphamide] within 30 days).

Definitions

Major infections included pneumonia or bacteremia. Pneumonia was diagnosed on the basis of radiographic pulmonary infiltrates and a quantitative bacterial culture of bronchoalveolar lavage demonstrating at least 10^4 colony-forming units per milliliter, as previously defined.[21] An episode of clinically significant bacteremia was defined as signs or symptoms of infection (fever, leukocytosis) and isolation of a bacterial pathogen from at least 1 blood culture. Bacteremia (single positive blood cultures) due to coagulase-negative staphylococci and other known common blood culture contaminants, including diphtheroids and *Bacillus* species, was excluded. The Acute Physiology and Chronic Health Evaluation (APACHE) II score and the Injury Severity Score were calculated within 24 hours of admission to the ICU, as previously described.[22,23] The term *immunocompetent* was used to describe patients lacking evidence of immunosuppression.

CMV Assays

Antibodies to CMV indicating prior CMV infection were assessed using a commercial enzyme immunoassay kit for detection of total antibodies to CMV (Abbott CMV Total AB EIA, Abbott Laboratories, Abbott Park, Illinois). The assay was performed and interpreted according to manufacturer recommendations. Cytomegalovirus DNA was quantified in stored plasma samples using a previously described real-time PCR assay.[24] DNA extraction was performed on 200 µL of plasma using a QIAamp DNA blood kit (Qiagen Inc, Valencia, California). Then, 100 µL of Tris (10 mM, pH 8.0) was used to elute the DNA, and 10 µL of the DNA was used for each PCR reaction.

The PCR conditions were 50°C for 2 minutes and 95°C for 2 minutes, followed by 45 cycles of 95°C for 20 seconds and 60°C for 1 minute. Each 50 µL of PCR mixture contained a 400-nM concentration of primers, 5 µL of 10× buffer II (Perkin-Elmer Cetus, Waltham, Massachusetts), 10 mM of magnesium chloride, 17.5 nM of TaqStart antibody (Mountain View, California), 1.25 U of AmpliTaq (Perkin-Elmer Cetus), 0.05 U of uracil-DNA-glycosylase, 8% glycerol, and 60 nM of 6-carboxy-x-rhodamine. To ensure that negative results were not due to nonspecific inhibition of the PCR assay, each PCR also contained internal positive control EXO DNA (5000 copies/reaction), primers, and probes. All negative CMV

PCR results required detection of EXO DNA. One positive control with 5000 copies of CMV DNA was coprocessed with specimens to ensure DNA recovery. To monitor for false-positive results, specimens were processed in parallel with aliquots of 1 × phosphate-buffered saline. Polymerase chain reactions without DNA also were included in each PCR run. Polymerase chain reactions were run in duplicate, with results deemed positive if both reactions were positive; results that were positive-negative were deemed indeterminate and repeated. Quantitative PCR levels are reported as copies per milliliter of plasma.

Statistical Analysis

Patient characteristics are summarized using percentages or median and range values. Cumulative incidence estimates for CMV viremia considered death or discharge from the hospital as competing risk events. In a landmark analysis of patients still hospitalized by 30 days after admission, probability of discharge after day 30 was calculated for patients who had reactivated CMV prior to day 30 and those who had not using cumulative incidence estimates with death considered a competing risk event. This specific time point for the landmark analysis was chosen because all patients had equal follow-up assessments of CMV reactivation, because virtually all patients who ever had CMV reactivation had done so by 30 days, and because it took into consideration a biologically relevant time lag for CMV effects. Log-rank tests were used to compare the hazards of discharge between groups. Proportions of days transfused or ventilated were calculated by summing the number of days the patient was transfused or ventilated by the total number of days followed up, to a maximum of 30 days for the composite end-point analysis.

Logistic regression models were used to identify risk factors for CMV reactivation and for the composite end point of continued hospitalization or death by day 30. Odds ratios (ORs) and 95% confidence intervals (CIs) are reported. Potential risk factors for CMV reactivation included age, race/ethnicity, sex, unit, baseline APACHE II score, baseline transfusion receipt, and baseline ventilator use. Potential risk factors for the composite end point included the aforementioned as well as major infection, CMV viral load measurements, and the proportion of hospitalized days spent transfused or ventilated. Risk factors that were univariately significant at $P < .10$ were considered for entry into multivariable models, which were limited to 3 factors because of the number of events.

The primary interest was the association between viral load and length of stay (LOS); thus, we categorized patients as remaining hospitalized longer than each of 4 time points: 14, 28, 42, and 56 days after admission. Since the covariate effects on the outcome of continued hospitalization longer than 14 days could be different than those on continued hospitalization longer than, for example, 42 days, we used partial proportional odds models to estimate odds of increased LOS past each consecutive time point. The proportional odds model[25,26] constrains the ORs for explanatory variables to be the same across outcome time points, whereas the partial proportional odds model allows the impact of some factors to vary across outcome time points while other factors maintain a constant effect.[27] We selected the partial proportional odds model as a means to evaluate the impact of CMV viral load on LOS.

We modeled CMV viral load in 2 ways: the average area under the curve (AUC) to reflect all follow-up and the 7-day moving average to reflect a shorter window of follow-up. With longitudinal measurements for each patient, we used these methods to smooth the viral load peaks and nadirs. The average AUC of CMV was calculated for each day of follow-up by summing each patient's CMV PCR measurements and dividing by the number of days followed up thus far. The 7-day moving average was calculated for each day of follow-up by summing the CMV PCR measurements over the previous 7 days and calculating the average value. For example, on day 7, the 7-day moving average would be the average of viral load measurements on days 1 through 7; the moving average on day 8 would average the measurements on days 2 through 8; on day 9, it would average the measurements on days 3 through 9; and so on. The average AUC, on the other hand, accumulates across all days followed up: on day 7, the average AUC would be the average of viral load measurements on days 1 through 7; on day 8, the average AUC would be the average of the measurements on days 1 through 8; and on day 9, it would be the average of viral loads on days 1 through 9. Therefore, each patient's viral load measurements were cumulated to reflect short-term and long-term averages while still contributing multiple data points.

Since each patient contributed observations from multiple time points to the analysis, we used generalized estimating equations with robust sandwich variance estimates to appropriately account for intrapatient correlations.[25]

Multivariable models were limited to 3 factors because of number of events or patients. All reported *P* values are 2-sided and $P < .05$ was considered significant. SAS software, version 9.1 (SAS Institute Inc, Cary, North Carolina) was used for all analyses, and figures were created with GraphPad Prism, version 4.03 for Windows (GraphPad Software, San Diego, California).

RESULTS

Study Population

A total of 221 patients were initially screened for inclusion in the study, and 101 were excluded on the basis of a negative CMV serologic result (n=78), death or discharge within 72 hours of admission (n=8), inability to provide informed consent (n=9), or other miscellaneous reasons (n=6), leaving 120 patients who comprised the study population. The characteristics of the study population stratified by ICU are shown in TABLE 1. Forty patients were enrolled each in the MICU and TICU and 20 patients each in the BICU and CICU. The primary composite end

point of continued hospitalization or death by 30 days occurred in 45 of 120 patients (38%).

Incidence and Quantitation of CMV Reactivation (Viremia)

The incidence of CMV viremia stratified by ICU is shown in TABLE 2. A total of 1954 samples were tested from the 120 enrolled patients, with a median of 11 (range, 1-89) samples tested per patient. The cumulative incidence of CMV viremia at any level and at greater than 1000 copies/mL, stratified by ICU, and for the entire cohort is shown in FIGURE 1. The cumulative incidence estimate of CMV viremia at any level was 33% (39/120; 95% CI, 24%-41%). Among patients in whom viremia ever developed, 37 of 39 (95%) did so within the first 30 days after admission to the ICU and half within the first 12 days (range, 3-57 days to first detectable viremia). The cumulative incidence estimate of CMV viremia at greater than 1000 copies/mL was 20% (24/120; 95% CI, 13%-28%), occurring at a median of 26 days (range, 9-56 days). The cumulative incidence estimates of CMV viremia at 30 days at any level were 0.45 (95% CI, 0.23-0.67), 0.15 (95% CI, 0-0.31), 0.25 (95% CI, 0.12-0.38), and 0.38 (95% CI, 0.22-0.53) and at greater than 1000 copies/mL were 0.05 (95% CI, 0-0.23), 0.05 (95% CI, 0-0.15), 0.15 (95% CI, 0.04-0.26), and 0.18 (95% CI, 0.08-0.32), respectively, in the BICU, CICU, MICU, and TICU.

Table 1. Characteristics of the Study Population

Characteristics	Overall (n = 120)	Burn ICU (n = 20)	Cardiac ICU (n = 20)	Medical ICU (n = 40)	Trauma ICU (n = 40)
Age, median (range), y	52 (18-90)	46 (19-80)	60 (42-90)	54 (19-80)	42 (18-87)
Male sex, No. (%)	73 (61)	14 (70)	13 (65)	23 (58)	23 (58)
White race, No. (%)	94 (78)	18 (90)	15 (75)	28 (70)	33 (83)
APACHE II score, median (range)	21 (7-36)	20 (11-33)	16 (7-34)	28 (10-36)	20 (11-30)
Transfusion within 24 h of admission, No. (%)	5 (4)	0	0	2 (5)	3 (8)
Mechanical ventilation use at admission, No. (%)	93 (78)	17 (85)	9 (45)	29 (73)	38 (95)
Major infection, No. (%)	41 (34)	15 (75)	1 (5)	11 (28)	14 (35)
Hospital length of stay, median (range), d	17 (2-181)	55 (8-181)	7 (2-41)	13 (4-94)	18 (6-86)
ICU length of stay, median (range), d	10 (1-126)	43 (8-126)	5 (1-18)	9 (3-55)	10 (3-56)
Deceased by 30 d postenrollment, No. (%)	10 (8)	2 (10)	5 (25)	2 (5)	1 (3)
Hospitalized at 30 d postenrollment, No. (%)	35 (29)	17 (85)	1 (5)	6 (15)	11 (28)
In ICU at 30 d postenrollment, No. (%)	20 (17)	13 (65)	0	2 (5)	5 (13)

Abbreviations: APACHE, Acute Physiology and Chronic Health Evaluation; ICU, intensive care unit.

Table 2. CMV Reactivation as Assessed by PCR

CMV Variable	Overall (n = 120)	Burn ICU (n = 20)	Cardiac ICU (n = 20)	Medical ICU (n = 40)	Trauma ICU (n = 40)
CMV viremia at any level, No. (%)	39 (33)	11 (55)	3 (15)	10 (25)	15 (38)
CMV viremia at >1000 copies/mL, No. (%)	24 (20)	9 (45)	1 (5)	6 (15)	8 (20)
CMV viremia at >10 000 copies/mL, No. (%)	11 (9)	4 (20)	0	4 (10)	3 (8)
Maximum CMV load, median (range), $\log_{10}$ PCR copies	3.3 (1.8-5.5)	3.9 (2.5-5.5)	2.4 (1.8-3.7)	3.4 (2.3-4.8)	3.1 (2.1-4.5)
Days to first detectable CMV viremia, median (range)	12 (3-57)	19 (7-57)	15 (9-21)	8 (3-13)	11 (3-21)
Duration of viremia, median (range), d	17 (2-45)	20 (4-45)	4 (2-17)	18 (4-38)	14 (2-32)

Abbreviations: CMV, cytomegalovirus; ICU, intensive care unit; PCR, polymerase chain reaction.

Risk Factors for CMV Reactivation

Multivariable logistic regression analysis of factors associated with CMV viremia at any level is shown in TABLE 3. In multivariable models, male sex was associated with an increased risk of CMV reactivation. The APACHE II score at admission was not associated with an increased risk of subsequent CMV reactivation. The results were similar when a CMV viremia end point of greater than 1000 copies/mL was used, except that the baseline variables of ventilator use (adjusted OR, 8.5; 95% CI, 1.1-66.5; $P=.04$) and receipt of a transfusion (adjusted OR, 6.7; 95% CI, 1.1-42.7; $P=.05$) were associated with an increased risk of CMV reactivation at that level (data not shown).

Risk Factors for Death or Continued Hospitalization by 30 Days

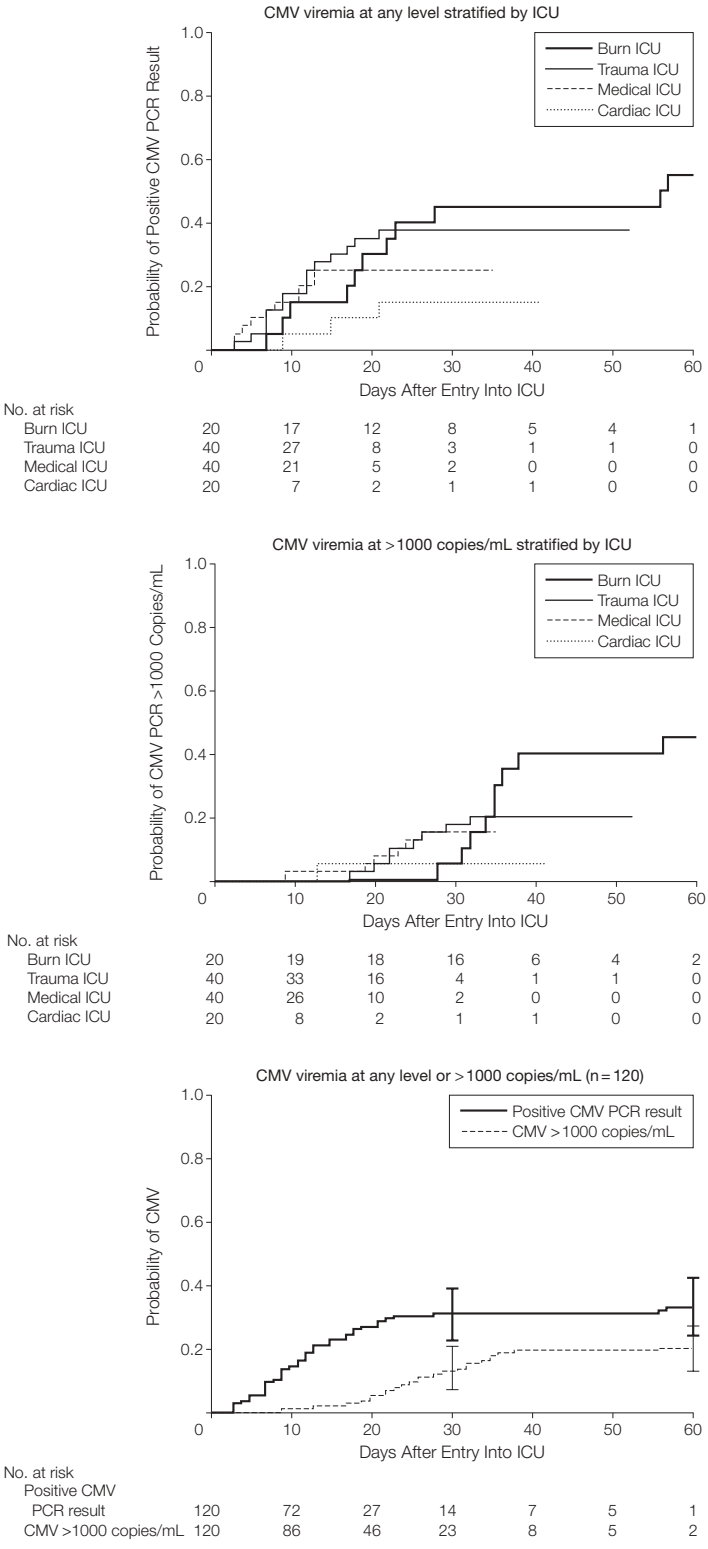
TABLE 4 shows the raw data for discharge, death, continued hospitalization, and CMV reactivation status of the cohort at days 7, 10, 15, 20, and 30 after admission to the intensive care unit. TABLE 5 shows the logistic regression univariable and multivariable analysis of factors associated with the composite end point of death or continued hospitalization by 30 days after admission to the ICU. After adjustment for other significant baseline or time-dependent variables, CMV reactivation assessed in any 1 of 4 ways (viremia at any level, viremia at >1000 copies/mL, maximum viremia in $\log_{10}$ copies/mL, or average AUC) was independently associated with death or continued hospitalization by 30 days. Fur-

thermore, there was a quantitative association such that the greater the amount of CMV reactivation, the greater the risk of continued hospitalization or death by 30 days. A similar association between CMV reactivation and death or continued hospitalization by the earlier time point of 15 days was evident (adjusted odds ratio for viremia at any level, 6.1; 95% CI, 1.7-21.7; $P=.005$; for maximum viremia in $\log_{10}$ copies/mL, 2.1; 95% CI, 1.2-3.7; $P=.007$; and for average AUC, 2.6; 95% CI, 1.1-6.2; $P=.03$).

FIGURE 2 shows the predicted probability of death or continued hospitalization by 30 days as a function of the average CMV AUC based on a logistic regression model. Each log increase in the average CMV AUC was associated with a 14% increase in the probability of death or continued hospitalization by 30 days. A similar analysis but using the composite end point of death or ICU (rather than total) hospitalization by 30 days yielded similar results: each of the CMV variables remained associated with death or ICU hospitalization by 30 days, with adjusted ORs for CMV viremia at any level of 5.7 (95% CI, 2.1-15.6; $P<.001$), for CMV viremia at greater than 1000 copies/mL of 4.6 (95% CI, 1.2-17.4; $P=.02$), for each $\log_{10}$ maximum CMV of 1.7 (95% CI, 1.2-2.4; $P=.002$), and for each $\log_{10}$ unit increase in average CMV AUC of 2.0 (95% CI, 1.3-3.1; $P=.003$).

Development of a major infection (nosocomial bacteremia or pneumonia) was associated with an increased hospital LOS (adjusted OR, 3.0; 95% CI, 1.1-8.4; $P=.04$). The association between CMV and death or continued hospitalization by 30 days after admission to the ICU remained significant when the analysis was restricted to the MICU and TICU cohorts only, with adjusted ORs for CMV viremia at any level of 7.3 (95% CI, 2.3-22.9; $P<.001$), for CMV viremia at greater than 1000 copies/mL of 32.4 (95% CI, 5.8-18.3; $P<.001$), for each $\log_{10}$ maximum CMV of 2.1 (95% CI, 1.5-3.0; $P<.001$); and for average CMV AUC of 2.7 (95% CI, 1.6-4.3; $P<.001$).

Figure 1. Cumulative Incidence of CMV Viremia



CMV indicates cytomegalovirus; ICU, intensive care unit; PCR, polymerase chain reaction. Error bars indicate 95% confidence intervals.

Risk Factors for Increased Hospital LOS

We used the variable of a 7-day CMV moving average to model the short-term effects of higher CMV viral load on the odds of staying longer in the hospital. In addition, we used the average CMV AUC to model the long-term effects; ie, the lasting effects of previous high viral loads on length of hospitalization. TABLE 6 shows that overall, a higher CMV moving average over the previous 7 days or average CMV AUC was associated with an increased hospital LOS. For example, for each $\log_{10}$ copies/mL increase in viral load over the previous 7 days, there was a 5.1-fold increased odds of being hospitalized for more than 14 days; similarly, for each $\log_{10}$ increase in viral load 7-day moving average, there was a 2.8-fold increased odds of being hospitalized for more than 28 days. This association did not remain significant for more extreme LOS (ie, for LOS greater than either 42 or 56 days). The average CMV AUC was also associated with an increased odds of continued hospitalization, regardless of when during the hospital stay this measurement was assessed (Table 6).

To assess the association between CMV reactivation and LOS in a group that was uniformly monitored for CMV reactivation, we performed a landmark analysis and assessed the cumulative incidence of time to discharge among the 35 patients who were still hospitalized by day 30 after admission. Patients were categorized as CMV reactivators if they tested positive by PCR prior to day 30. FIGURE 3 shows that the hazard of discharge is significantly greater in nonreactivators compared with reactivators ($P=.03$ by log-rank test). The median LOS after day 30 in reactivators (n=21) was 24 days (range, 3-64 days) compared with 10 days (range, 1-151 days) in nonreactivators (n=14).

Table 3. Risk Factors for CMV Reactivation[a]

Baseline Characteristic	OR (95% CI)	*P* Value	Adjusted OR (95% CI)	*P* Value
Age (10-y increments)	1.1 (0.9-1.4)	.43		
Intensive care unit				
Trauma	1 [Reference]		1 [Reference]	
Burn	2.0 (0.7-6.1)	.20	1.8 (0.6-5.7)	.30
Cardiac	0.3 (0.1-1.2)	.08	0.2 (0.1-1.0)	.06
Medical	0.6 (0.2-1.5)	.23	0.5 (0.2-1.4)	.20
Race				
White	1 [Reference]			
Other	0.9 (0.4-2.3)	.83		
Sex				
Female	1 [Reference]		1 [Reference]	
Male	3.6 (1.5-8.8)	.005	3.8 (1.5-9.5)	.005
APACHE II score quartile				
<16	1 [Reference]			
≥16 to <21	2.1 (0.5-8.6)	.29		
≥21 to <27.5	0.8 (0.2-3.9)	.83		
≥27.5	2.8 (0.7-11.1)	.15		
Transfusion				
No	1 [Reference]			
Yes	9.1 (1.0-84.7)	.05		
Mechanical ventilation				
No	1 [Reference]			
Yes	2.5 (0.9-7.3)	.09		

Abbreviation: APACHE, Acute Physiology and Chronic Health Evaluation.
[a]Odds ratios (ORs) and 95% confidence intervals (CIs) were estimated by logistic regression models.

Table 4. Raw Data for Status of Hospitalization, Mortality, and CMV Reactivation by Day After Admission

	Index Day				
	7	10	15	20	30
Discharged before, No.	20	33	46	61	75
Died before, No.	5	7	9	9	10
Continued hospitalization on, No.	95	80	65	50	35
Among patients still hospitalized on index day, No.					
Never CMV-reactivated	56	42	31	20	12
Reactivated before	11	16	22	23	21
Reactivated after	28	22	12	7	2
CMV 7-d moving average at index day, median (range), $\log_{10}$ copies/mL	0.5 (0.2-2.4)	0.7 (0.2-2.5)	0.6 (0-3.3)	1.2 (0-3.4)	2.4 (0-4.1)
P value[a]	<.001	<.001	.001	<.001	
Average CMV AUC at index day, median (range), $\log_{10}$ copies/mL	1.3 (0.2-2.4)	1.4 (0.6-2.6)	1.3 (0.3-3.1)	1.6 (0.3-3.6)	2.3 (0.9-3.5)
P value[b]	.001	<.001	.002	<.001	

Abbreviations: AUC, area under the curve; CMV, cytomegalovirus.
[a]*P* Value compares CMV 7-day moving average at index day with 7-day moving average at day 30 adjusted for intrapatient correlation using generalized estimating equations.
[b]*P* value compares average CMV AUC at index day with average AUC at day 30 adjusted for intrapatient correlation using generalized estimating equations.

COMMENT

Using a prospective, blinded study design and rigorous statistical analyses in a broad range of immunocompetent patients with critical illness, we demonstrated that reactivation of CMV occurs frequently and is independently and quantitatively associated with a clinically relevant end point of continued hospitalization or death by 30 days after admission to the ICU. Thus, we have identified a novel and potentially modifiable risk factor for death or prolonged hospitalization in critically ill patients.

Given the number, complexity, potential bidirectional relationships between CMV and other variables analyzed, and the time-varying nature of the end points, we used a variety of statistical methods to comprehensively assess the relationship between CMV and adverse clinical outcomes. These included use of partial proportional odds models, use of a novel parameter of 7-day moving average of CMV viral load throughout the hospital stay, and use of a composite end point of death or continued hospitalization by 30 days. In particular, use of the composite end point was objective, clinically relevant, and one that could be used as a primary end point in subsequent interventional studies of CMV prevention in this setting. Furthermore, the composite end point (rather than use of LOS alone) was used to reduce the potential impact that early deaths might have on assessment of the relationship between CMV reactivation and LOS.

Similarly, use of the partial proportional odds models allowed us to control for the observed relationship between LOS and onset of CMV reactivation, thereby allowing the relationship of CMV reactivation and subsequent LOS to be assessed throughout the hospital stay. In addition, given the concern that longer LOS would lead to a greater opportunity to detect CMV reactivation (and, thus, potentially lead to a spurious association between CMV reactivation and LOS), we performed a landmark analysis among those who were hospitalized for at least 30 days (a time point at which 95% of those who ultimately ever had reactivation of CMV had done so, and also a subset who all had a uniform duration of monitoring for CMV). As in the previous analyses, CMV reactivation was associated with longer durations of subsequent hospitalization compared with those who did not reactivate by day 30 (Figure 3). The association between CMV reactivation and prolonged hospitalization or death remained robust throughout all of the analyses.

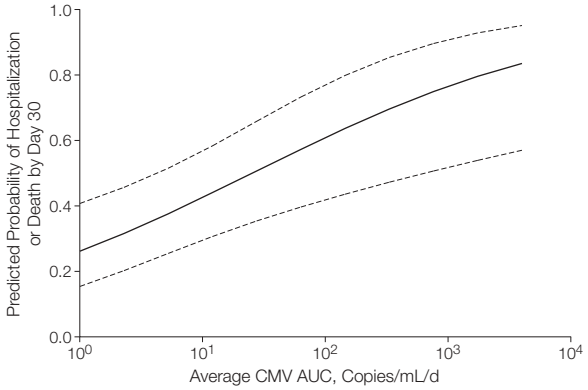
Thus, our data are consistent with the possibility that CMV reactivation is causally related to prolongation of hos-

Table 5. Risk Factors for Continued Hospitalization or Death by Day 30 After Admission to Intensive Care Unit[a]

	OR (95% CI)	*P* Value	Adjusted OR (95% CI)	*P* Value
Baseline variables				
Age (10-y increments)	1.1 (0.9-1.4)	.31		
Intensive care unit				
Trauma	1 [Reference]		1 [Reference]	
Burn	44.3 (5.3-370)	<.001	90 (8.3-980)[b]	<.001
Cardiac	1.0 (0.3-3.2)	>.99	2.6 (0.6-10.2)[b]	.18
Medical	0.8 (0.3-2.1)	.62	0.8 (0.3-2.2)[b]	.65
Race				
White	1 [Reference]			
Other	0.4 (0.2-1.2)	.09		
Sex				
Female	1 [Reference]			
Male	1.1 (0.5-2.3)	.88		
APACHE II score quartile				
<16	1 [Reference]			
≥16 to <21	1.0 (0.3-3.8)	.98		
≥21 to <27.5	1.0 (0.3-3.8)	.98		
≥27.5	1.6 (0.5-5.8)	.46		
Transfusion				
No	1 [Reference]			
Yes	2.4 (0.4-15.1)	.34		
Mechanical ventilation				
No	1 [Reference]		1 [Reference]	
Yes	4.5 (1.5-14.1)	.009	10.2 (1.9-55.5)[c]	.007
Hospital stay variables				
Major infection				
No	1 [Reference]		1 [Reference]	
Yes	4.8 (2.1-10.7)	<.001	3.0 (1.1-8.4)[d]	.04
CMV viremia at any level				
No	1 [Reference]		1 [Reference]	
Yes	4.6 (2.0-10.3)	<.001	4.3 (1.6-11.9)[d]	.005
CMV viremia at >1000 copies/mL				
No	1 [Reference]		1 [Reference]	
Yes	7.8 (2.0-29.7)	.003	13.9 (3.2-60.9)[d]	<.001
Maximum CMV load, $\log_{10}$ copies/mL	1.8 (1.4-2.3)	<.001	1.8 (1.3-2.4)[d]	<.001
Average CMV AUC, $\log_{10}$ copies/mL/d	1.8 (1.3-2.7)	.001	2.1 (1.4-3.2)[d]	<.001
Transfusion days (10% increments)	1.2 (0.8-1.7)	.43		
Ventilator days (10% increments)	1.2 (1.0-1.3)	.01	1.3 (1.1-1.7)[d]	.01

Abbreviations: APACHE, Acute Physiology and Chronic Health Evaluation; AUC, area under the curve; CMV, cytomegalovirus.
[a] Odds ratios (ORs) and 95% confidence intervals (CIs) were estimated by logistic regression models.
[b] Adjusted for baseline ventilation.
[c] Adjusted for intensive care unit.
[d] Adjusted for intensive care unit and baseline ventilation.

Figure 2. Predicted Probability of Death or Continued Hospitalization by Day 30 as a Function of Average CMV AUC, Adjusted for Unit and Baseline Ventilator Use



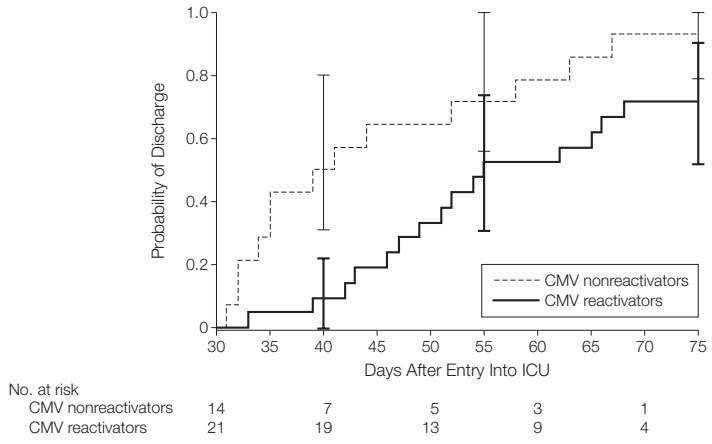
The predicted probabilities of death or continued hospitalization were estimated from a logistic regression model of average cytomegalovirus (CMV) area under the curve (AUC), adjusted for unit and baseline ventilator use. The dashed curves indicate 95% confidence intervals. Average CMV AUCs were 0 for 83 patients; within the range of 0.5 to 1.50 $\log_{10}$ copies/mL for 7 patients; within the range of 1.51 to 2.50 $\log_{10}$ copies/mL for 18 patients; and within the range of 2.51 to 3.6 $\log_{10}$ copies/mL for 12 patients.

Table 6. Partial Proportional Odds Model Results for the Association of 7-Day CMV Moving Average or Average CMV AUC With Hospital Length of Stay, Adjusted for Unit

	Length of Stay, d							
	≥14		≥28		≥42		≥56	
	OR (95% CI)	*P* Value	OR (95% CI)	*P* Value	OR (95% CI)	*P* Value	OR (95% CI)	*P* Value
CMV 7-day moving average per $\log_{10}$ copies/mL of viral load	5.1 (2.9-9.1)	<.001	2.8 (1.5-5.4)	.002	1.7 (0.8-3.5)	.18	1.1 (0.5-2.5)	.78
Average CMV AUC per $\log_{10}$ copies/mL of viral load	3.2 (2.1-4.7)	<.001	3.5 (2.2-5.7)	<.001	3.2 (1.9-5.4)	<.001	3.1 (1.7-5.6)	<.001

Abbreviations: AUC, area under the curve; CI, confidence interval; CMV, cytomegalovirus; OR, odds ratio.

Figure 3. Cumulative Incidence of Hospital Discharge After 30 Days According to CMV Reactivation Status



CMV indicates cytomegalovirus; ICU, intensive care unit. Error bars indicate 95% confidence intervals.

pital stay in this clinical setting, and this contention is also supported by animal studies.[28] However, we emphasize that an observational study design cannot establish causality and that the data presented herein are also consistent with the possibility that CMV reactivation is simply a marker (rather than a determinant) for prolonged hospital stay. We did not find an association between severity of illness (as assessed by the APACHE II score) and risk of CMV reactivation, thereby diminishing the likelihood that CMV reactivation was simply a surrogate marker of illness severity.

The only definitive means of differentiating between a role of CMV as a cause vs marker for adverse clinical outcomes is by means of a randomized controlled trial of antiviral prophylaxis in this clinical setting. Given the major importance of the clinical problem, the availability of generally safe and well-tolerated antiviral agents with activity against CMV, combined with the data regarding CMV incidence and endpoint estimates generated in this study, we believe that a randomized, placebo-controlled trial of antiviral prophylaxis is both warranted and feasible and should be a priority among studies to improve the outcomes of patients with critical illness.

The mechanism(s) underlying the observed association between CMV and adverse clinical outcomes are not defined in the present study. One possibility is direct CMV pathogenicity, and this has previously been reported in the setting of otherwise immunocompetent patients with critical illness but appears to be uncommon.[16] Another possibility is that 1 or more CMV indirect effects are responsible for the observed association between CMV reactivation and adverse clinical outcomes. Cytomegalovirus-mediated immunosuppression leading to an increased risk of secondary infections[2-5] and CMV-mediated lung injury[28,29] are the most plausible mechanisms in this clinical setting. In support of these possibilities are in vitro and animal model experimental data,[28,30,31] clinical obser-

vational studies[1,9,32] and the demonstration that antiviral therapy reduces these effects in animal models[28] and in controlled clinical trials in certain patient populations.[2-5] Larger prospective studies that include laboratory investigations will be necessary to define the mechanism(s) underlying the association of CMV reactivation with adverse clinical outcomes in patients with critical illness.

There were several strengths of the present study, including the prospective, blinded design, inclusion of a broad range of critically ill patients, use of quantitative CMV assessments, and use of comprehensive statistical analyses with an adequate number and frequency of clinically relevant end points. This is the largest study conducted to date, and the results are statistically robust. It is reassuring that factors previously reported to be associated with increased LOS (eg, bacteremia, pneumonia) were confirmed to be associated with LOS in the present study.[33-35]

The study also had potential limitations. Monitoring for CMV reactivation was not performed in discharged patients. It is possible, though we think it is unlikely, that some discharged patients may have first reactivated CMV after hospital discharge, which would make it more difficult to conclude that CMV had a biologically significant effect. There is also a potential concern that the association between CMV reactivation and prolonged hospital stay could, in part, be related to a greater opportunity to detect CMV reactivation in those with longer hospital stays (ie, "circular reasoning").

However, the known biological time lag of CMV effects in other settings, the quantitative nature of the association demonstrated in the present study, and the consistent finding of the association between CMV reactivation and prolonged LOS in the landmark analysis and partial proportional odds models (both of which directly addressed the time-dependent nature of CMV reactivation) all support the contention that CMV reactivation was associated with prolongation of hospital stay rather than a spurious finding. We are careful to emphasize that our study design (or any observational study design) cannot prove causality between CMV and adverse clinical outcomes in this setting. Rather, we consider these results to be hypothesis generating and to provide useful background data, which, when combined with prior investigations, provide the rationale for performing definitive interventional studies. Even though a strong association between CMV reactivation and prolonged LOS was identified, the mechanism(s) underlying this association could not be defined in this study. Also, not all variables previously reported to be associated with an increased LOS were examined in the present study.

In summary, we have demonstrated an independent and quantitative association between CMV viral load and prolonged LOS in a broad range of immunocompetent patients with critical illness. These findings, combined with data from prior investigations, provide a strong rationale for a randomized controlled trial of antiviral prophylaxis in this clinical setting.

Author Contributions: Dr Limaye had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.
Study concept and design: Limaye, Bulger, Neff, Gibran, Corey, Boeckh.
Acquisition of data: Limaye, Huang, Santo Hayes, Boeckh.
Analysis and interpretation of data: Limaye, Kirby, Rubenfeld, Leisenring, Gibran, Boeckh.
Drafting of the manuscript: Limaye, Kirby, Leisenring.
Critical revision of the manuscript for important intellectual content: Limaye, Kirby, Rubenfeld, Leisenring, Bulger, Neff, Gibran, Huang, Santo Hayes, Corey, Boeckh.
Statistical analysis: Limaye, Kirby, Rubenfeld, Leisenring, Neff.
Obtained funding: Limaye, Boeckh.
Administrative, technical, or material support: Limaye, Bulger, Gibran, Huang, Santo Hayes, Corey, Boeckh.
Study supervision: Limaye, Leisenring, Boeckh.
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Anatomical dissection gives the human mind an opportunity to compare the dead with the living, things severed with things intact, things destroyed with things evolving, and opens up the profoundness of nature to us more than any other endeavor or consideration.

—Johann Wolfgang von Goethe (1749-1832)

