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Serial Cerebrospinal Fluid CMV DNA Monitoring During Extended Induction Therapy for HIV-Associated Central Nervous System Cytomegalovirus Infection: A Retrospective Cohort Study

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Abstract

Background: Central nervous system (CNS) cytomegalovirus (CMV) infection remains a devastating complication among people with HIV (PWH), with historical mortality exceeding 80% despite standard 2–3-week antiviral induction. Although plasma CMV DNA predicts mortality, cerebrospinal fluid (CSF) viral load dynamics and their therapeutic implications remain poorly characterized.

Methods: We conducted a retrospective review of 33 PWH with confirmed CNS CMV infection (CD4 <200 cells/μL) admitted to a tertiary hospital in Nanjing, China, between March 2019 and August 2024. All patients received induction therapy (94% ganciclovir + foscarnet) with serial CSF CMV DNA monitoring. Clinical features, virologic dynamics, treatment responses, and 182-day outcomes were examined. Delayed CSF clearance was defined as CSF CMV DNA detectable at the last available CSF observation (never cleared).

Results: Median baseline CD4 count was 11 cells/μL (IQR 4–34); 87.9% had CD4 <50 cells/μL. Extended induction (median 6 weeks, IQR 4–8) achieved 72.7% 182-day survival. CSF CMV clearance was substantially slower than plasma clearance: 63.6% remained CSF PCR-positive (>500 copies/mL) at week 2 vs. 42.4% with detectable

plasma viremia. Non-survivors had higher baseline CSF CMV loads (4.9 vs. 3.9 log_{10} copies/mL, P = 0.075) and pronounced CSF inflammation. Grade ≥3 hematologic toxicity occurred in 54.5%. Neurological symptom burden was prognostically relevant: patients with ≥2 of four neurological symptoms at presentation had significantly worse 182-day survival than those with ≤1 symptom (42.9% vs. 94.7%; log-rank P = 0.0010).

Conclusions: In this cohort, patients receiving CSF-monitored extended induction therapy had a 72.7% 182-day survival rate. Compartmentalized viral kinetics suggest that CSF-specific monitoring may identify patients who require prolonged therapy, though randomized trials are warranted. Symptom burden may help identify patients at highest risk.

Keywords: central nervous system; cytomegalovirus; cerebrospinal fluid viral load; HIV; antiviral therapy; extended induction

List of abbreviations

aHR: Adjusted hazard ratio

AIDS: Acquired immunodeficiency syndrome

ART: Antiretroviral therapy

CD4: Cluster of differentiation 4

CD8: Cluster of differentiation 8

CI: Confidence interval

CMV: Cytomegalovirus

CNS: Central nervous system

CSF: Cerebrospinal fluid

CSF: Cerebrospinal fluid

DNA: Deoyribronucleic acid

HIV: Human immunodeficiency virus

HR: Hazard ratio

IQR: Interquartile range

IRIS: Immune reconstituton infammatory syndrome

LDH: Lactate dehydrogenase

mNGS: Metagenomic next-generation sequencing

OR: Odds ratio

PCR: Polymerase chain reaction

PWH: People with HIV

RNA: Ribonucleic acid

WBC: White blood cell

Background

Cytomegalovirus (CMV) represents a prevalent opportunistic infection among people living with HIV (PWH), particularly among individuals with advanced immunosuppression (CD4 counts <50 cells/mm³) [1, 2]. In this high-risk population, CMV manifestations include retinitis, gastrointestinal involvement, pneumonitis, and less commonly, central nervous system (CNS) disease [2–4]. Notably, CNS CMV infection,

though accounting for only 1–2% of AIDS-defining conditions, carries substantially worse prognosis compared to other forms of CMV disease, with mortality rates exceeding 80% in historical cohorts [5–7].

The high mortality associated with CNS CMV infections stems from multiple factors. Diagnostic delays frequently occur due to non-specific clinical presentation, which may mimic HIV encephalopathy, cerebral toxoplasmosis, or lymphoma [8, 9]. Therapeutic challenges persist due to: (1) the absence of consensus treatment protocols; (2) dose-limiting toxicities of antiviral agents; and (3) uncertainty regarding optimal treatment duration [7, 9, 10]. Historical data, primarily from a 2010 Brazilian cohort of 19 patients, reported 19% 6-month survival despite combination therapy [7] while guidelines recommending standard 2–3-week induction have shown limited efficacy in this population [11]. More recent data from the late ART era suggest potentially improved outcomes [10], though evidence remains limited to small retrospective series.

While plasma CMV DNA levels have been established as an independent predictor of mortality in PWH [2], the prognostic value and therapeutic implications of CSF viral load dynamics remain poorly characterized. To address this knowledge gap, we conducted a retrospective observational study to assess the clinical utility of serial CSF viral load monitoring in guiding personalized treatment duration for CNS CMV infections.

Methods

Study Population

This retrospective cohort study analyzed 33 PWH with confirmed CNS CMV infection admitted to the Second Hospital of Nanjing between March 2019 and August 2024. Inclusion criteria were: (1) HIV-1 infection confirmed by Western blot; (2) acute neurological manifestations; (3) CD4 count <200 cells/μL; (4) concomitant CSF CMV DNA positivity with supportive neuroimaging findings. Exclusion criteria included prior antiviral treatment for CMV initiated elsewhere or discordant mNGS/PCR results. This study is reported in accordance with the STROBE guidelines for cohort studies.

Study size: The sample size (n = 33) represents all eligible patients meeting the inclusion criteria during the 66-month study period at this single referral center. No a priori sample size calculation was performed; this is a convenience sample reflecting the rarity of confirmed CNS CMV infection.

Setting: The Second Hospital of Nanjing is a tertiary referral center with a dedicated HIV care unit and serves as a regional HIV treatment hub, receiving referrals from Nanjing and neighboring cities. As part of routine care, all HIV patients with suspected CNS infection underwent CSF CMV DNA testing on admission. Follow-up continued until death, loss to follow-up, or study end (February 2025); all surviving patients had a minimum potential follow-up of 182 days.

During the study period, 42 patients with suspected CNS CMV infection were screened; 9 were excluded because CSF mNGS detected CMV but confirmatory CSF CMV PCR was negative (reflecting the higher sensitivity of mNGS for low-burden CMV DNA),

yielding 33 analyzable patients (Additional file 1). No eligible patient refused participation or was lost to follow-up before the primary endpoint.

Diagnostic Workup

Routine CSF analysis included cell count, protein, glucose, chloride, and cryptococcal antigen testing. CSF mNGS was performed to identify co-pathogens as previously described [12]. All patients underwent brain contrast-enhanced MRI; CT was performed if contraindicated. Exposed-CMV IRIS was diagnosed using the INSHI consensus criteria: (1) CMV disease diagnosed before ART initiation; (2) ART initiated within the preceding 6 months; (3) clinical worsening after initial improvement on antiviral therapy; and (4) an inflammatory CSF profile not attributable to other causes. CSF and plasma CMV viral load were quantified using a real-time PCR assay with a limit of detection of 500 copies/mL; values below the limit of detection were treated as undetectable. Baseline CMV load was measured within 72 hours of admission and before initiation of induction therapy.

Antiviral Therapy

Induction therapy consisted of intravenous ganciclovir (5 mg/kg twice daily) and/or foscarnet (60 mg/kg three times daily). Treatment duration was at the treating physician's discretion based on serial CSF CMV DNA monitoring, with CSF CMV viral load and plasma CMV viral load reassessed approximately every 2 weeks during induction and a general goal of achieving CSF viral clearance before transitioning to maintenance. No

formal protocol specified minimum or maximum treatment duration; the median achieved induction duration was 6 weeks (IQR 4–8).

Data Collection and Outcomes

Study variables and definitions: The primary outcome was all-cause mortality within 182 days of treatment initiation, and CSF CMV viral kinetics was evaluated as the key exposure and virologic outcome. The primary exposure variable was delayed CSF clearance, defined as CSF CMV DNA detectable (>500 copies/mL) at the last available follow-up CSF observation (i.e., never cleared); serial CSF CMV viral load and plasma CMV viral load was reassessed approximately every 2 weeks during induction. A priori-selected potential confounders included age, sex, baseline CD4 count, plasma HIV RNA level, ART status at admission (naive vs. experienced vs. virologic failure), baseline plasma CMV viral load, and concurrent AIDS-defining illnesses. Secondary outcomes included adverse events (DAIDS v2.1) and 24-week immune recovery. All variable definitions and categorization boundaries were established before data analysis.

Baseline data were extracted from standardized case-report forms and electronic medical records, including age, sex, comorbidities, AIDS-defining illnesses, CD4 count, HIV RNA, plasma and CSF CMV viral loads, ART status, and neurological symptoms. Data completeness and missingness are described in the Results; missing values were handled by complete-case analysis.

Statistical Analysis

CSF CMV viral loads were log10-transformed before correlation and regression analyses to approximate normality. Continuous variables were categorized only at pre-specified clinically meaningful thresholds declared before analysis (CSF chloride <120 vs. ≥120 mmol/L; baseline CSF CMV >100,000, >300,000, and >500,000 copies/mL); no data-driven cutoff optimization was performed.

Continuous variables were summarized as medians (IQR) and compared using the Mann–Whitney U test; categorical variables were compared using Fisher's exact test. Correlation between plasma and CSF CMV viral loads was assessed on log10-transformed values (Spearman's rank correlation). Survival was estimated using the Kaplan–Meier method. Delayed CSF clearance was defined as CSF viral load detectable (>500 copies/mL) at the last available follow-up CSF observation. Exploratory Cox proportional hazards regression assessed the association between delayed CSF clearance and 182-day mortality, adjusted for baseline CSF CMV viral load and CD4 count; the proportional hazards assumption was tested using Schoenfeld residuals (P = 0.42). To address survivor bias, a landmark sensitivity analysis restricted the cohort to patients alive on day 28. Because only 9 deaths occurred, Firth's penalized logistic regression with two pre-specified covariates (baseline CSF CMV viral load and CD4 count) was performed as a sensitivity analysis. Linearity of continuous variables was assessed; missing data were handled by complete-case analysis; propensity-based methods were not feasible given the sample size. All the analyses were performed with

the statistical software packages R (http://www.R-project.org, The R Foundation) and Free Statistics software versions 2.3.

Bias Handling

Efforts to address potential bias: Selection bias was minimized by enrolling consecutive eligible patients during the study period. To reduce ascertainment bias, CMV testing was performed as part of a standardized diagnostic protocol for all HIV patients with suspected CNS infection rather than at the discretion of individual clinicians. Information bias was limited by the use of objective, PCR-quantified laboratory endpoints rather than subjective assessments. However, confounding by indication remains an inherent limitation of the observational design, because treatment duration was guided by CSF viral load dynamics rather than randomization; this is addressed further in the Limitations section.

Ethical Considerations

The study was approved by the Ethics Committee of the Second Hospital of Nanjing (Approval No. 2024-LS-ky076). Written informed consent was obtained from all participants or their legal representatives.

Results

Baseline Characteristics

Participant flow: Of 42 screened patients, 9 were excluded because CSF mNGS detected CMV but confirmatory CSF CMV PCR was negative, reflecting the higher sensitivity of mNGS for low-burden CMV DNA detection; the remaining 33 patients were analyzable

(Additional file 1). Baseline characteristics are described below; no patient was lost to follow-up within the 182-day window.

The cohort included 33 PWH, predominantly male (87.9%) with median age 43 years (IQR 31–51). Twenty-six patients (78.8%) were newly diagnosed with HIV. At admission, 20 (60.6%) were ART-naïve, 7 (21.2%) had been on ART for <12 weeks, 1 (3.0%) for ≥12 weeks, and 5 (15.2%) had virologic failure. Median CD4 count was 11 cells/μL (IQR 4–34), with 87.9% having CD4 <50 cells/μL. CMV end-organ diseases included retinitis (36.4%) and gastrointestinal disease (12.1%; Table 1). Data completeness: baseline demographic, CD4 count, plasma HIV RNA, plasma CMV DNA, CSF CMV DNA, CSF chloride, CSF WBC, CSF LDH, CSF protein, and CSF glucose were complete for all 33 patients (100%). A total of 162 serial CSF CMV measurements were available (median 4 per patient, IQR 2-6);31 patients (93.9%) had at least one follow-up CSF test after baseline, and the remaining 2 patients contributed baseline measurements only (one of whom died before repeat lumbar puncture could be performed).

Table 1 Clinical characteristics and laboratory results of CNS cytomegaloviral infection in 33 PWH

Characteristics	Total (n = 33)	Survivors (n = 24)	Non-survivors (n = 9	*P* value
Age, years	43.0 (31.0-51.0)	44.5 (30.5-54.0)	41.0 (37.0-45.0)	0.824
18~59	29 (87.9)	20 (83.3)	9 (100)	
≥60	4 (12.1)	4 (16.7)	0 (0)	
Sex				0.052
Male	29 (87.9)	23 (95.8)	6 (66.7)	

Female	4 (12.1)	1 (4.2)	3 (33.3)	
Modes of HIV transmission				0.19
Heterosexual	21(63.6)	13(54.2)	8(88.9)	
Homosexual	11(33.3)	10(41.7)	1(11.1)	
Other	1(3.0)	1(4.2)	0(0)	
Other CMV EOD[1]				
CMV_retinitis[2]	12 (36.4)	9 (37.5)	3 (33.3)	0.999
GI diseases[3]	4 (12.1)	3 (12.5)	1 (11.1)	0.999
Other AIDS-define illness[2]				
Candida esophagitis	14 (42.4)	11 (45.8)	3 (33.3)	0.698
Pneumocystis pneumonia	13 (39.4)	9 (37.5)	4 (44.4)	0.999
CM/cryptococcal septicemia[4]	8 (24.3)	6 (25.0)	2 (22.2)	0.597
NTM infection	8 (24.2)	7 (29.2)	1 (11.1)	0.394
Non-Hodgkin lymphoma	3 (9.1)	2 (8.3)	1 (11.1)	0.999
Tuberculosis	2 (6.1)	1 (4.2)	1 (11.1)	0.517
Toxoplasmic encephalitis	2 (6.1)	2 (8.3)	0 (0)	0.999
Kaposi's sarcoma	1 (3.0)	0 (0)	1 (11.1)	0.273
Underlying diseases				0.999
Syphilis	8 (24.2)	6 (25.0)	2 (22.2)	0.999
Hypertension	3 (9.1)	3 (12.5)	0 (0)	0.545
Diabetes	2 (6.1)	1 (4.2)	1 (11.1)	0.477
HBV co-infection	2 (6.1)	1(4.2)	1 (11.1)	0.477
HCV co-infection	1 (3.0)	1 (4.2)	1 (11.1)	0.999
ART status				0.81
Not on ART	20 (60.6)	13 (54.2)	7 (77.8)	
Effective ART < 12 w	7 (21.2)	6 (25.0)	1 (11.1)	
Effective ART > 12 w	1 (3.0)	1 (4.2)	0 (0)	
Ineffective ART	5 (15.2)	4 (16.7)	1 (11.1)	
Ad-ART time, day	16.0 (8.0, 26.0)	14.5 (7.8, 21.0)	37.0 (26.0, 38.0)	0.126
CD4 cell count, cells/ mL[5]	11 (4.0-34.0)	20.0 (4.0-40.0)	8.0 (2.0-14.0)	0.217
<50	29 (87.9)	20 (83.3)	8 (88.9)	
50~100	1 (3.0)	1 (4.2)	0 (0)	
≥100	3 (9.1)	2 (8.3)	1 (11.1)	
IRIS	4(12.1)	3(12.5)	1 (11.1)	0.999
Plasma HIV RNA, log10 copies/ m	5.1 (4.5-5.6)	5.2 (4.2-5.7)	5.2 (4.8-5.3)	0.903
Plasma CMV load, log10 copies/ m	4.5 (3.7-5.3)	4.4 (3.6-5.8)	4.5 (4.4-5.0)	0.746
CSF CMV load, log10 copies/mL	4.3 (3.3- 5.0)	3.9 (3.2-4.8)	4.9 (4.8-5.4)	0.075

Categorical data are presented as number (percentage). Continuous data are presented as median (interquartile range, IQR).

[1]It was difficult to etiologically diagnose CMV pneumonia in the majority of our patients, therefore the prevalence of

CMV pneumonia was not calculated in this study.

[2]CMV retinitis was diagnosed based on characteristic retinal changes on ophthalmoscopic examination.

[3]The diagnosis of GI diseases based on upper and/or lower gastrointestinal symptoms plus macroscopic mucosal lesions plus CMV documented in tissue by histopathology.

[4]CM/cryptococcal septicemia group included 5 patients with cryptococcal meningitis and 3 patients with cryptococcal septicemia but without any evidence of cryptococcal meningitis.

[5]Four patients of unmasking IRIS. IRIS occurred in two patients with cryptococcal meningitis, one patient with toxoplasmic encephalitis and one patient with lymphoma after ART. The CD4 cell count, HIV load, and CMV DNA levels before and after IRIS, as well as the duration of ART, are presented in Additional file 7.

Abbreviations: CMV, cytomegalovirus; CSF, cerebrospinal fluid; PWH, people with human immunodeficiency virus; EODs, end-organ diseases; GI, gastrointestinal; CM, cryptococcal meningitis; NTM, nontuberculous mycobacteria; HCV, Hepatitis C virus; HBV, Hepatitis B virus; ART, antiretroviral therapy.

Neurologic symptoms and CSF Findings

Neurologic symptoms associated with outcome: disturbance of consciousness was present in 7/9 non-survivors [77.8%] versus 4/24 survivors [16.7%], P = 0.002; nausea in 7/9 [77.8%] versus 8/24 [33.3%], P = 0.047; vomiting in 7/9 [77.8%] versus 6/24 [25.0%], P = 0.011; and urinary retention/sphincter dysfunction in 5/9 [55.6%] versus 4/24 [16.7%], P = 0.073. CSF findings were also associated with mortality: higher CSF WBC (median 44.0 vs. 3.5 × 10^6/L, P = 0.005) and CSF LDH (130.0 vs. 25.5 IU/L, P = 0.013), consistent with a more severe acute meningoencephalitis phenotype (Table 2).

Table 2 Clinical findings, cerebrospinal fluid, MRI imaging findings, treatment and prognosis of CNS cytomegaloviral infection in 33 PWH

Characteristics	Total (n = 33)	Survivors (n = 24)	Non-survivors (n = 9)	*P* value
Symptoms				

Fever	24 (72.7)	16 (66.7)	8 (88.9)	0.384
Headache	20 (60.6)	15 (62.5)	5 (55.6)	0.999
Progressive bilateral leg weakness	16 (48.5)	12 (50.0)	4 (44.4)	0.999
Nausea	15 (45.5)	8 (33.3)	7 (77.8)	0.047
Slow reaction	14 (42.4)	9 (37.5)	5 (55.6)	0.442
Vomiting	13 (39.4)	6 (25.0)	7 (77.8)	0.011
Disturbance of consciousness	11 (33.3)	4 (16.7)	7 (77.8)	0.002
Stiff neck	11 (33.3)	9 (37.3)	2 (22.2)	0.681
Anal sphincter dysfunction/urinary retention	9 (27.3)	4 (16.7)	5 (55.6)	0.073
Seizure	5 (15.2)	2 (8.3)	3 (33.3)	0.111
Memory decline	5 (15.2)	4 (16.7)	1 (11.1)	0.999
Cerebrospinal fluid analysis				
White blood cell count,106/L	7.0 (2.0-44.0)	3.5 (1.8-9.5)	44.0 (8.0-476.0)	0.005
< 8	18 (54.5)	16 (66.7)	2 (22.2)	
≥8	15 (45.5)	8 (33.3)	7 (77.8)	
Proteins, mg/L	504.9 (408.0-1035.0)	456.5 (373.0-800.0)	984.5 (619.4-1407.8)	0.052
< 500	16 (48.5)	14 (58.3)	2 (22.2)	
500-1000	8 (24.3)	5 (20.8)	3 (33.3)	
≥1000	9 (18.2)	5 (20.8)	4 (44.4)	
Lactate dehydrogenase, IU/L	29 (18.0-130.0)	25.5 (17.5-35.2)	130.0 (75.0-393.0)	0.013
Glucose, mmol/L	2.4 (2.0-3.1)	2.6 (2.2-3.0)	2.2 (1.4- 4.2)	0.442
< 2.5	17 (51.5)	11 (45.8)	6 (66.7)	
≥2.5	16 (48.5)	13 (54.2)	3 (33.3)	
Chloride, mmol/L	120.0 (115.0-126.0)	120.0 (116.8-125.8)	119.0 (113.0-126.0)	0.331
< 120	15 (45.5)	10 (41.7)	5 (55.6)	
≥120	18 (54.5)	14 (58.3)	4 (44.4)	
Adenosine deaminase, U/L	5.0 (1.0-6.5)	3.0 (0.8-6.2)	6.0 (5.0-6.5)	0.067
MRI/CT abnormal[1]	28(84.8)	21(87.5)	7(77.8)	0.597
Anti-CMV treatment combination[2]	31(93.9)	23(95.8)	8(88.9)	0.563
Anti-CMV time, week	6.0 (4.0, 8.0)	6.0 (4.0, 8.0)	4.0 (3.0, 6.0)	0.119
Number of follow-up tests				
Plasma CMV load	3.0 (2.0, 4.0)	3.0 (3.0, 4.0)	2.0 (0.0, 3.0)	0.036

CSF CMV load	3.0 (2.0, 6.0)	4.0 (2.0, 6.0)	2.0 (1.0, 3.0)	0.069

Categorical data are presented as number (percentage). Continuous data are presented as median (interquartile range, IQR).

[1] A total of 32 patients underwent cranial MRI, while only one had cranial CT scans.

[2]One patient received ganciclovir alone, and one was treated exclusively with foscarnet sodium due to granulocyte deficiency.

Abbreviations: PMN: polymorphonuclear neutrophils; MRI: magnetic resonance imaging.

Baseline CSF chloride was available in all 33 patients: median 120 mmol/L (IQR 115–126), with 15 (45.5%) below 120 mmol/L; across 162 serial samples, the median was 122 mmol/L (IQR 118–125). Low baseline CSF chloride (<120 mmol/L) was not associated with baseline CSF CMV viral load (P = 0.73), CSF LDH (P = 0.30), or mortality (Fisher's exact P = 0.70); the association with CSF WBC was borderline (P = 0.05) (Additional file 3).

Viral Load Dynamics

Plasma and CSF CMV viral loads showed a moderate correlation (r = 0.53, r^2 = 0.28, P = 0.002; Figure 1). Using a >500 copies/mL threshold applied to the most recent test within each week, CSF CMV remained detectable at week 2 in 63.6% vs. 42.4% in plasma; at week 4, 45.5% vs. 18.2%; and at week 6, 30.3% of patients remained CSF-positive (vs. 12.1% in plasma). Six patients maintained low-level CSF CMV viral load (>500 copies/mL) between weeks 8–12. Individual patient CSF viral load trajectories with sample size annotations at each time point are shown in Figure 2.

Predictors of Mortality

In exploratory Cox regression, delayed CSF clearance (detectable at the last CSF observation, never cleared) was not significantly associated with mortality (HR 2.62, 95% CI 0.70–9.76, P = 0.152; log-rank P = 0.14;); after adjustment for baseline CSF CMV viral load and CD4 count, the association remained directionally consistent but non-significant (aHR 1.97, 95% CI 0.50–7.78, P = 0.334; Table 3). The alternative definition (detectable at any CSF test later than 6 weeks) was not interpretable, because no patient with a CSF test after 6 weeks died (0/9 versus 9/24, log-rank P = 0.043), consistent with survivorship bias. A Kaplan–Meier curve is presented in Figure 3, with full model details in Additional file 4A. In Firth's penalized logistic regression, baseline CSF CMV viral load showed an OR of 1.69 (95% CI 0.84–3.38) per log10 increase (P = 0.138) and CD4 count was not significantly associated with mortality (OR 0.99, 95% CI 0.97–1.02, P = 0.503) (Additional file 6). CSF CMV load threshold analyses did not dichotomize outcomes: mortality was 1/6 (16.7%) above versus 8/27 (29.6%) below the pre-specified 300,000 copies/mL threshold (Fisher's exact P = 1.00); alternative thresholds of 100,000 and 500,000 copies/mL also yielded no significant separation (P = 0.65 and P = 1.00, respectively) (Additional file 7).

In univariable analyses, higher CSF WBC (HR 2.16 per $\log_{10}$ cell/μL, 95% CI 1.27–3.67, P = 0.004) and CSF LDH (HR 3.79 per $\log_{10}$ IU/L, 95% CI 1.43–10.03, P = 0.007) were associated with increased mortality, and disturbance of consciousness (HR 9.35, 95% CI 1.93–45.29, P = 0.005) and vomiting (HR 7.30, 95% CI 1.51–35.42, P = 0.014) were the

300 symptom predictors most strongly associated with mortality. In multivariable models
301 (Table 3), CSF LDH (aHR 11.01 per $\log_{10}$ IU/L, 95% CI 2.37–51.19, P = 0.002), CSF
302 WBC (aHR 4.59 per $\log_{10}$ cell/μL, 95% CI 1.79–11.79, P = 0.002), vomiting (aHR 10.32,
303 95% CI 1.90–55.94, P = 0.007), disturbance of consciousness (aHR 9.38, 95% CI 1.71–
304 51.53, P = 0.010) and nausea (aHR 7.48, 95% CI 1.48–37.75, P = 0.015) were also
305 independently associated with mortality, whereas female sex showed a large but
306 imprecise adjusted hazard (aHR 8.59, 95% CI 1.75–42.10, P = 0.008), based on only 4
307 female patients (3 deaths).
308
309 **Table 3. Univariable and multivariable Cox regression for 182-day all-cause**
310 **mortality (n = 33, events = 9)**
311

Variable	Univariable HR (95%CI)	*P value*	Multivariate HR (95%CI)	*P value*
Female sex	6.49 (1.54,27.33)	0.011	8.59 (1.75–42.10)	0.008
Age, years	0.99 (0.943,1.044)	0.764		
CD4 count, cell/μL	0.99 (0.96,1.02)	0.371	0.99 (0.96–1.02)	0.497
Plasma HIV RNA, log10 copies/ mL	1.11 (0.70,1.77)	0.645		
Baseline plasma CMV load, $\log_{10}$ copy/mL	1.02 (0.63,1.65)	0.938	0.79 (0.46–1.35)	0.387
Baseline CSF CMV load, $\log_{10}$ copy/mL	1.43 (0.88–2.34)	0.150	1.43 (0.86–2.37)	0.167
Delayed CSF clearance (never cleared)	2.62 (0.70–9.76)	0.152	1.97 (0.50–7.78)	0.334
Disturbance of consciousness	9.35 (1.93,45.29)	0.005	9.38 (1.71–51.53)	0.010
Anal sphincter dysfunction/urinary retention	4.13 (1.1,15.47)	0.035	3.38 (0.88–13.02)	0.076
Nausea	5.35 (1.11,25.85)	0.037	7.48 (1.48–37.75)	0.015
Vomiting	7.3 (1.51,35.42)	0.014	10.32 (1.90–55.94)	0.007
CSF WBC, $\log_{10}$ cell/μL	2.16 (1.27–3.67)	0.004	4.59 (1.79–11.79)	0.002

CSF LDH, log_{10} IU/L	3.79 (1.43–10.03)	0.007	11.01 (2.37–51.19)	0.002

Survival time in days (censored at 182). Delayed CSF clearance defined as CSF CMV load detectable at the last CSF observation (never cleared), consistent with Figure 3. CSF WBC and CSF LDH were log_{10}-transformed because of right skew; untransformed values are shown in Table 2 using rank-based tests. Multivariable model adjusted for baseline CSF CMV viral load and CD4 count (pre-specified); with 9 events, only the exposure plus two pre-specified confounders were entered. Univariable models were not adjusted for multiple comparisons. HR: hazard ratio; CI: confidence interval.

Landmark Sensitivity Analysis

To address survivor bias, a landmark analysis restricted to the 31 patients alive at day 28 was performed; the 2 patients who died before day 28 were excluded. Among landmark survivors, 182-day survival was 78.6% in patients who continued therapy for >6 weeks (n = 14) versus 76.9% in those who completed 4–6 weeks (n = 13); the difference was not statistically significant (log-rank P = 0.853), indicating that the observed association between longer therapy duration and survival was substantially attenuated after conditioning on survival to day 28, consistent with survivor bias. Patients who completed <4 weeks of induction (n = 4) were excluded from this comparison.

Regarding neurological symptom burden, a score (0–4) summing the four neurological symptoms recorded during induction (disturbance of consciousness, vomiting, nausea, and urinary retention) was computed: patients with a score of ≥2 had significantly worse overall survival than those with a score of ≤1 (182-day survival 42.9% [8 deaths among 14] versus 94.7% [1 death among 19]; log-rank P = 0.0010; Figure 4B). The score summed the four neurological symptoms with the strongest univariable associations with mortality

(disturbance of consciousness, vomiting, nausea; all $P < 0.05$) plus urinary retention, retained on clinical grounds despite $P = 0.073$. This analysis is hypothesis-generating.

Adverse Events and Outcomes

Grade ≥3 hematologic toxicity occurred in 54.5% (anemia: 54.5% [18/33]; neutropenia: 24.2% [8/33]). Management included packed red blood cell transfusion (36.4%), erythropoietin support (15.2%), and granulocyte colony-stimulating factor for grade ≥3 neutropenia (12.1%). Dose reduction of ganciclovir occurred in 3 patients (9.1%) due to neutropenia. No patient discontinued therapy due to toxicity alone. Four patients (12.1%) developed exposed-CMV IRIS (3 paradoxical and 1 unmasking) (Additional file 7). During 182-day follow-up, 72.7% survived. Median time to death was 50 days (range 10–151).

Discussion

This study describes survival outcomes in a contemporary cohort of CNS CMV infection in PWH treated with CSF-monitored extended induction therapy. The observed 72.7% 182-day survival exceeds historical reports of 19–32% survival in pre-2010 cohorts [7, 10]. Our prior work (Fang et al. 2023, CID) established plasma CMV viral load as a mortality predictor in hospitalized PWH but did not examine CSF viral dynamics or CNS-specific outcomes [2]. The present study extends these findings by focusing exclusively on CNS CMV infection with longitudinal CSF monitoring, representing a distinct clinical question with different diagnostic and therapeutic implications. Additionally, two recent Chinese cohort studies have reported persistently poor CSF viral clearance and high

mortality in HIV-associated CMV encephalitis despite combination antiviral therapy [24, 25].

The temporal dissociation between CSF and plasma CMV clearance (63.6% CSF-positive at week 2 vs. 42.4% plasma-positive, with persistent separation through week 6 (30.3% vs. 12.1%)) suggests compartmentalized replication [15, 16]. The modest correlation ($r = 0.53$, $r^2 = 0.28$) indicates ~72% of CSF variance is independent of plasma levels. This aligns with suboptimal CNS ganciclovir penetration [14], supporting the rationale for prolonged therapy. Elevated CSF LDH and WBC in non-survivors suggest CMV-driven blood–brain barrier disruption via mechanisms such as matrix metalloproteinase-9 activation, as characterized in HIV-associated neurocognitive disorders and CMV-driven CNS pseudotumor formation [17–19]. The more frequent disturbance of consciousness, vomiting, and nausea among non-survivors, together with their higher CSF WBC and LDH, is consistent with a severe acute meningoencephalitis phenotype and CMV-driven blood–brain barrier disruption. These symptom–CSF associations are exploratory and were not adjusted for multiple comparisons; they should be interpreted as hypothesis-generating rather than as independent prognostic markers.

Two actionable hypotheses emerge: (1) CSF PCR positivity beyond 2 weeks may identify patients needing extended induction, analogous to CMV retinitis protocols [20, 21]; and (2) threshold-based approaches could mitigate CNS pharmacologic sanctuary effects. Maribavir, which achieves a CSF-to-plasma ratio of 94% in primate models, may warrant

evaluation in HIV-associated CNS CMV infection, although clinical data in this setting remain limited [22, 23].

Limitations

The present study has several important limitations that should be considered when interpreting these findings. First, these results are most applicable to hospitalized PWH with advanced immunosuppression (CD4 <50 cells/μL) and confirmed CNS CMV infection in settings with access to serial CSF CMV PCR testing and combined ganciclovir/foscarnet induction therapy. Caution is warranted in extrapolating to outpatient settings, resource-limited environments without routine CSF CMV PCR capacity, or populations with different CMV seroprevalence and ART coverage profiles. Second, the small sample size (n = 33, 9 events) from a single center limited precision and generalizability and precluded comprehensive multivariable adjustment (primary analyses were univariable). Third, survivor bias (median time to death 50 days; range 10–151 days) was addressed in part by a 4-week landmark sensitivity analysis that indicated the association between longer therapy and survival may be partly explained by survival itself. Fourth, confounding by indication was inherent since treatment duration was physician-directed. Fifth, the exploratory nature of the Cox regression and threshold analyses should be noted. Sixth, the absence of a contemporaneous fixed-duration control group precludes causal attribution of the survival benefit. Finally, potential selection and measurement biases inherent to retrospective data cannot be excluded.

Conclusions

In this cohort, patients receiving CSF-monitored extended induction therapy had a 72.7% 182-day survival rate. Compartmentalized viral kinetics support the potential utility of CSF-specific monitoring. Randomized trials comparing fixed-duration versus CSF-guided strategies are needed to establish causality.

Declarations

Ethics approval and consent to participate: The study protocol was approved by the Ethics Committee of the Second Hospital of Nanjing (Approval No. 2024- LS-ky076). Written informed consent was obtained from all participants or their legally authorized representatives.

Consent for publication: Written informed consent was obtained from all participants or their legally authorized representatives.

Availability of data and materials: Due to patient privacy restrictions and the terms of the institutional ethics approval, the data underlying this study cannot be publicly shared. Data may be available from the corresponding author upon reasonable request, subject to institutional approval.

Competing interests: The authors declare that they have no competing interests.

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Authors' contributions: Mingxia Fang: Conceptualization, Methodology, Data curation, Formal analysis, Writing – original draft. Zheng Qian: Conceptualization, Data curation. Yaling Chen, Yun Chi, Cong Cheng and Hongxia Wei: Data curation, Investigation.

Zhiliang Hu: Conceptualization, Supervision, Project administration, Writing – review & editing.

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Additional files

Additional file 1: STROBE flow diagram. Participant selection flow diagram showing screening, exclusion, and final cohort.

Additional file 2: STROBE checklist. Completed STROBE checklist for cohort studies.

Additional file 3: Baseline CSF chloride values and correlation with CSF CMV viral load. CSF chloride was available in all 33 patients (median 120 mmol/L, IQR 115–126); 15 (45.5%) had chloride below 120 mmol/L.

Additional file 4: Exploratory Cox proportional hazards regression for delayed CSF clearance. Univariable: HR 2.62 (95% CI 0.70–9.76, P = 0.152); adjusted for baseline CSF CMV viral load and CD4 count: aHR 1.97 (95% CI 0.50–7.78, P = 0.334).

Additional file 5: Firth's penalized logistic regression. Baseline CSF CMV viral load: OR 1.69 (95% CI 0.84–3.38, P = 0.138); CD4 count: OR 0.99 (95% CI 0.97–1.02, P = 0.503).

Additional file 6: CMV viral load threshold analyses. Mortality by CSF CMV load thresholds (300,000, 100,000, and 500,000 copies/mL): no significant separation at any threshold.

Additional file 7: IRIS patient details. Clinical characteristics of four patients who developed exposed-CMV immune reconstitution inflammatory syndrome.

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Figure Legends

Figure 1. Associations between baseline CSF CMV DNA load and immunovirological parameters.

Spearman rank correlations between baseline CSF CMV DNA and plasma CMV DNA, CD4+ T-cell count, CD8+ T-cell count, and plasma HIV RNA. r and P values are shown in each panel.

[Figure 1 - see separate high-resolution file]

Figure 2. CSF CMV DNA kinetics during extended induction therapy (n = 23).

Individual trajectories in 23 patients with ≥2 serial CSF measurements. Red dashed line: limit of detection (500 copies/mL). Numbers above the x-axis indicate the number of patients with a CSF measurement in each time band.

[Figure 2 - see separate high-resolution file]

Figure 3. Kaplan–Meier survival curves by delayed CSF clearance status (n = 33). Delayed clearance: CSF CMV DNA detectable at the last CSF observation (n = 12); cleared: last CSF observation undetectable (n = 21). Log-rank P = 0.137. Survival time in days from the start of induction therapy, censored at 182 days. Numbers at risk are shown

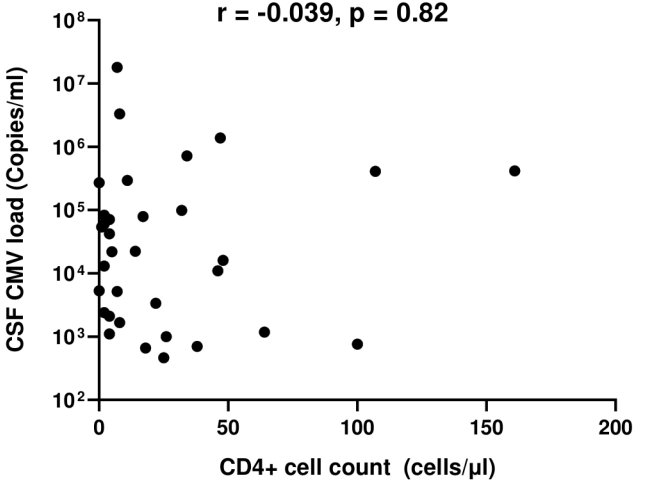
below the x-axis. The alternative Methods-text definition (detectable at any test >6 weeks) is not shown because no patient with a CSF test after 6 weeks died (0/9 versus 9/24), consistent with survivorship bias.

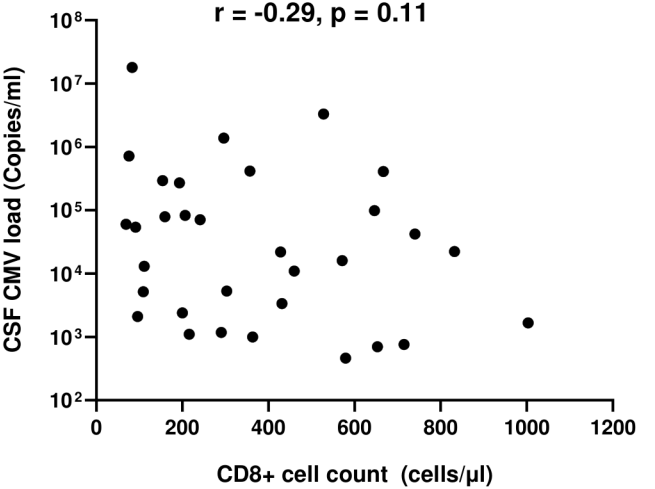
[Figure 3 - see separate high-resolution file]

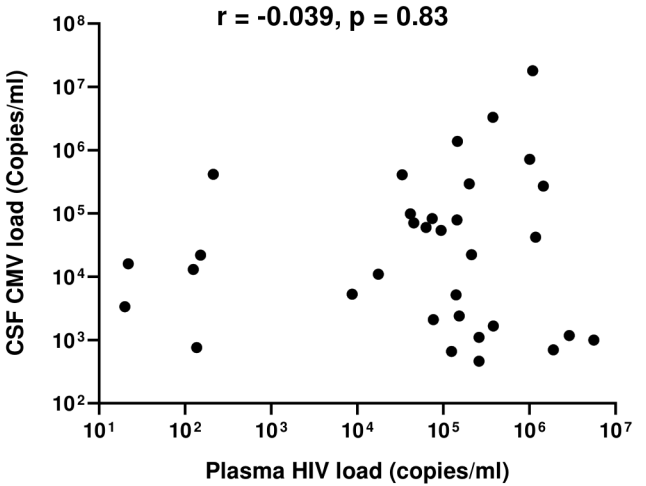
Figure 4. Overall survival and Kaplan–Meier curves by neurological symptom-burden score (n = 33).

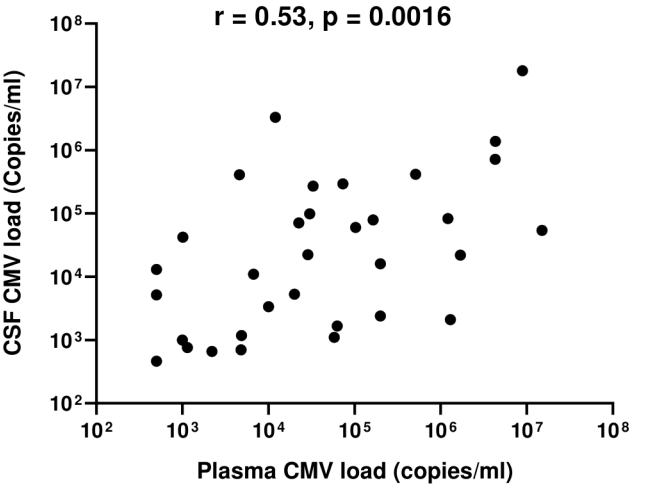
(A) Overall survival of the entire cohort (182-day survival 72.7%, 9 deaths among 33; numbers at risk below the panel). (B) Survival stratified by symptom-burden score ≥2 (n = 14; 8 deaths; 182-day survival 42.9%) versus ≤1 (n = 19; 1 death; 94.7% survival); log-rank P = 0.0010. The score counts four neurological symptoms present at presentation (disturbance of consciousness, vomiting, nausea, and urinary retention), dichotomized at ≥2. Survival time measured from induction therapy start (days), censored at 182 days; numbers at risk are shown below each panel.

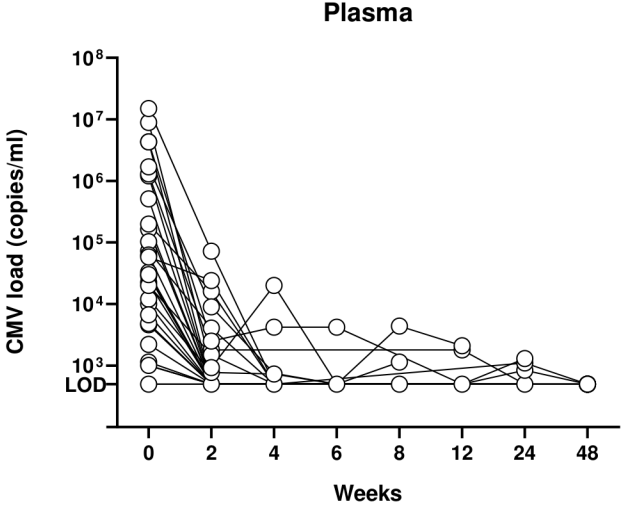
[Figure 4 - see separate high-resolution file]

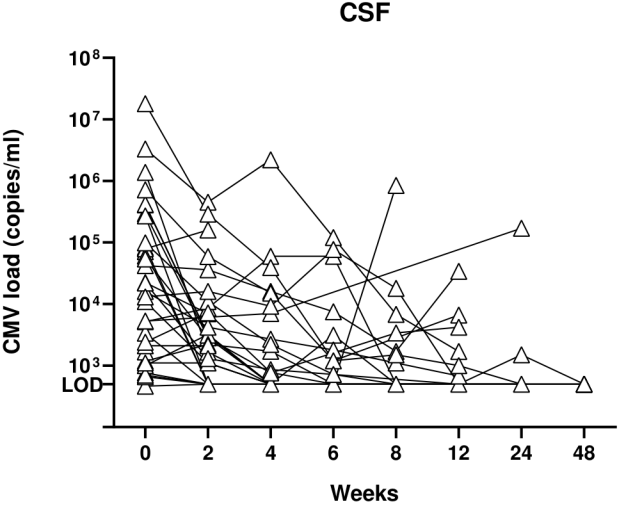

r = -0.039, p = 0.82
CSF CMV load (Copies/ml)
CD4+ cell count (cells/µl)

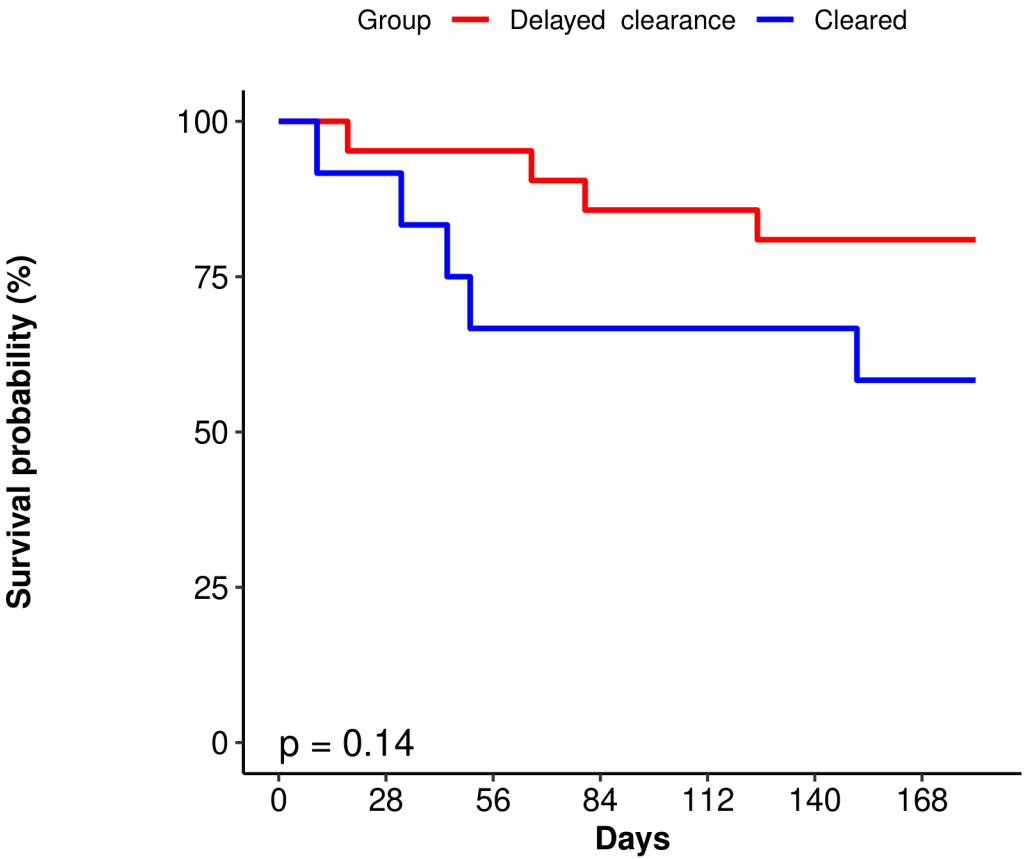

r = -0.29, p = 0.11
CSF CMV load (Copies/ml)
CD8+ cell count (cells/µl)


r = -0.039, p = 0.83
CSF CMV load (Copies/ml)
Plasma HIV load (copies/ml)


r = 0.53, p = 0.0016
CSF CMV load (Copies/ml)
Plasma CMV load (copies/ml)

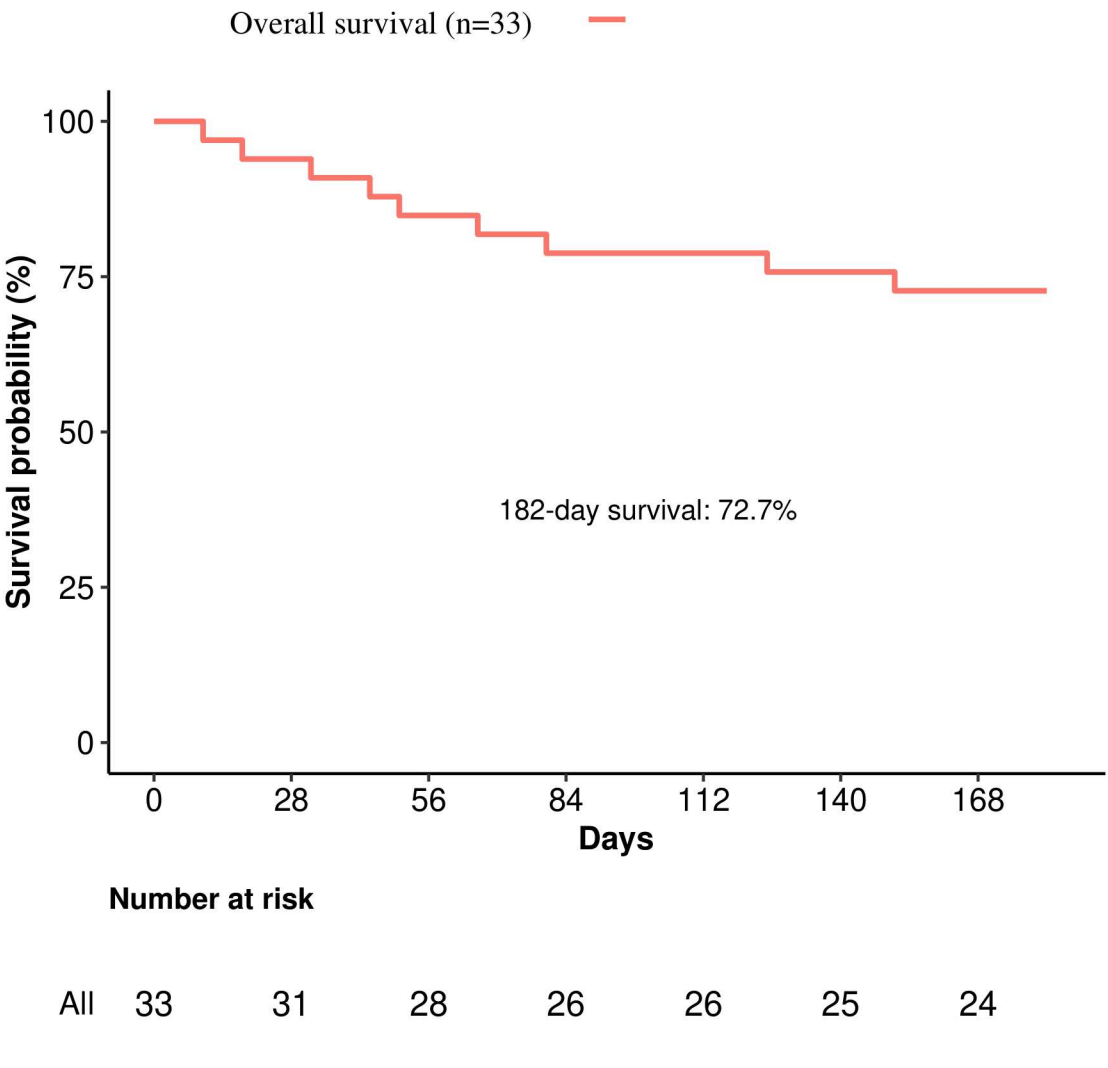

Plasma
CMV load (copies/ml)
10^8
10^7
10^6
10^5
10^4
10^3
LOD
0
2
4
6
8
12
24
48
Weeks

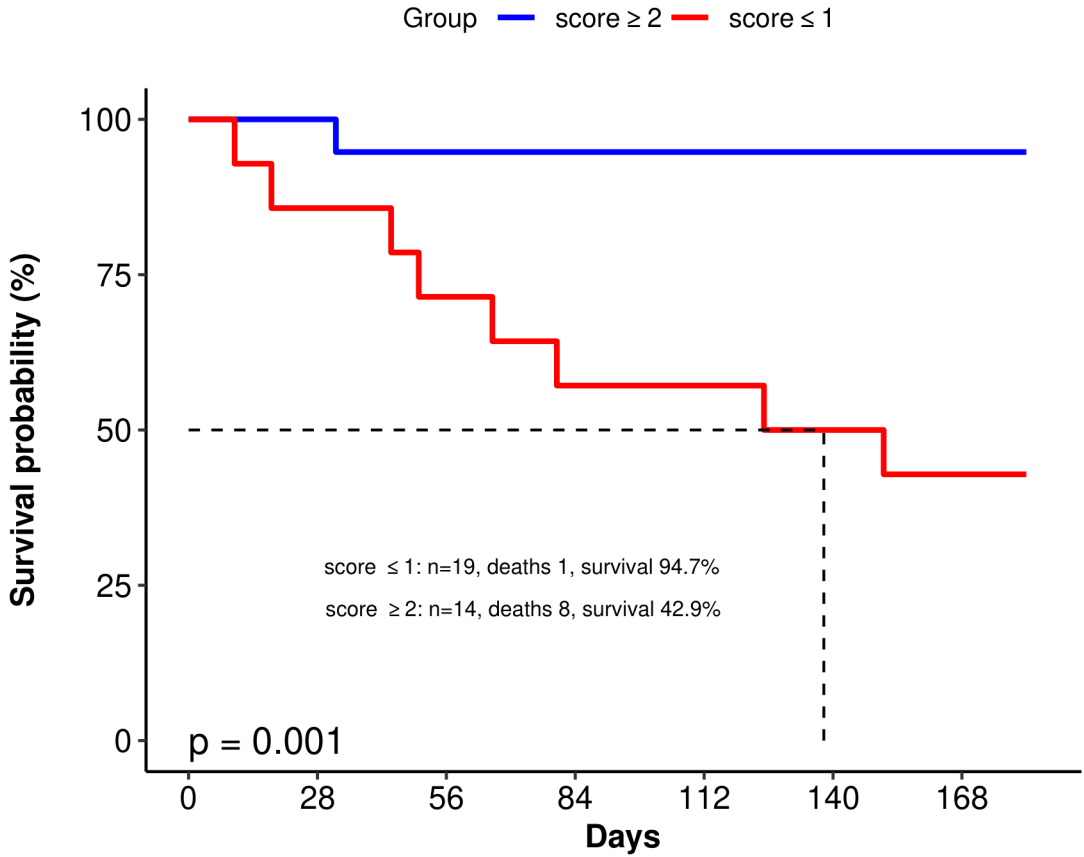

CSF
CMV load (copies/ml)
10^8
10^7
10^6
10^5
10^4
10^3
LOD
0
2
4
6
8
12
24
48
Weeks



Number at risk

Delayed clearance	21	20	20	18	18	17	17
Cleared	12	11	8	8	8	8	7


Overall survival (n=33)
100
75
50
25
0
Survival probability (%)
182-day survival: 72.7%
0
28
56
84
112
140
168
Days
Number at risk
All
33
31
28
26
26
25
24



Number at risk

score ≥ 2	19	19	18	18	18	18	18
score ≤ 1	14	12	10	8	8	7	6

Supplementary Files

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