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Cytomegalovirus reactivation and acute and chronic complications in children with cerebral malaria: a prospective cohort study

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Abstract

Background Virus co-infection or reactivation may modify the host response during cerebral malaria. Cytomegalovirus (CMV) DNAemia has been associated with increased morbidity and mortality in adults with sepsis; however, the impact of CMV DNAemia on adverse outcomes in children with cerebral malaria is unknown.

Methods Clinical, physiological, and neurocognitive outcomes were compared in children aged 18 months to 12 years with cerebral malaria (N = 242) based on the presence or absence of CMV DNAemia 24 h after admission. The primary study outcome was subsequent in-hospital mortality. Secondary outcomes included the presence of acute kidney injury, neurocognitive impairment over a 2-year follow-up, and chronic kidney disease at the 1-year follow-up. Markers of platelet and endothelial cell activation and oxidative and nitrosative stress were measured to characterize the mechanisms by which CMV DNAemia might contribute to pathogenesis.

Results CMV DNAemia was present in 33 children with cerebral malaria (13.6%) 24 h after admission. CMV DNAemia was not significantly associated with mortality in this study. Children with CMV-DNAemia had a higher prevalence of acute kidney injury than those without CMV-DNAemia (59.4% vs. 38.6%, p = 0.03). There was no difference in the prevalence of chronic kidney disease or long-term neurocognitive impairment based on the presence of DNAemia. CMV DNAemia was associated with elevated plasma levels of P-selectin, angiopoietin-1, asymmetric dimethylarginine, and platelet counts.

Conclusions In children with cerebral malaria, CMV DNAemia is associated with acute kidney injury but not in-hospital mortality, chronic kidney disease, or long-term neurocognitive impairment.

Keywords Cerebral malaria, Cytomegalovirus, Acute kidney injury, P-selectin, Platelet activation, Angiopoietin-1, Asymmetric dimethylarginine

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Background

Malaria is a leading infectious cause of mortality globally, with 263 million estimated cases and 597,000 deaths in 2023 [1]. Approximately 95% of deaths occur in the World Health Organization African region, and 76% of these deaths occur in children aged < 5 years [1]. Cerebral malaria (CM) is the most severe malarial complication. Mortality rates approach 20% and there is an increased risk of long-term neurocognitive impairment in survivors [2]. The pathogenesis of CM is not completely understood, but parasite sequestration in the brain microvasculature, host inflammatory response, oxidative stress, and endothelial activation contribute to the disease [3]. The impact of co-infection, including the reactivation of latent infections, during a clinical malaria episode is understudied.

Malaria may occur with viral coinfection or reactivation, including cytomegalovirus (CMV), a human herpesvirus [4, 5]. CMV seropositivity in adults ranges from 60–100% with a higher prevalence in low-income and middle-income countries [6]. During primary infection, CMV invades endothelial cells and is often asymptomatic or mildly symptomatic in immunocompetent hosts. Following infection, CMV establishes life-long latency in haematopoietic progenitor cells and possibly endothelial cells [7], with intermittent reactivation throughout the lifetime of the host, particularly during times of stress, infection, or immunosuppression.

CMV DNAemia in critically ill adults has been associated with clinical complications including acute kidney injury (AKI), longer duration of mechanical ventilation and hospitalization, and increased mortality [8, 9]. CMV has been associated with endothelial dysfunction [10] and oxidative stress [11], both of which occur in CM [12]. There is an absence of data on the prevalence of CMV DNAemia and its physiological and clinical complications in pediatric CM. The researchers postulated that CMV DNAemia would be associated with adverse clinical outcomes during the initial hospitalization and, if substantiated, that this association might correlate with longer-term disease sequelae.

Therefore, the prevalence of CMV DNAemia was investigated in a large cohort of children with CM and to examine the association of DNAemia with the early clinical outcomes of mortality and AKI and the long-term outcomes of chronic kidney disease (CKD) and neurocognitive impairment during the follow-up period. Markers of platelet and endothelial activation as well as oxidative and nitrosative stress were measured to elucidate mechanisms by which CMV might contribute to adverse clinical outcomes.

Methods

Study population

This cohort study was conducted at the Mulago National Referral and Teaching Hospital in Kampala, Uganda, from 2008–2013. Children between 18 months and 12 years of age were recruited from the Acute Care Unit at Mulago Hospital as previously described [13] and enrolled into either CM, severe malarial anaemia, or age-matched community children control groups. The present study included only children with CM. CM was defined based on the World Health Organization definition as follows: (1) coma (Blantyre coma score ≤ 2), (2) *Plasmodium falciparum* found on blood smear, and (3) no other known aetiology of coma. Exclusion criteria included (1) chronic medical condition requiring medical care, (2) known history of neurodevelopmental delay, or (3) prior history of cerebral palsy, coma, head trauma, or hospitalization for malnutrition, as described previously [13]. Children with CM or SMA were managed according to the Ugandan Ministry of Health treatment guidelines at the time of the study. These included intravenous quinine treatment followed by oral quinine for severe malaria while admitted and artemisinin combination therapy for outpatient follow-up therapy [13]. The primary study outcome was in-hospital mortality. The secondary outcomes included AKI on admission, CKD at 12-months, and neurocognitive outcomes measured at 24-months. Markers of platelet and endothelial activation and oxidative and nitrosative stress were measured to identify the mechanisms by which CMV DNAemia may contribute to pathogenesis.

Sample preparation

Glucose (Glucometer), haemoglobin (Hemocontrol), and lactate levels (Lactometer) were measured at enrolment. Approximately 5.5 mL of whole blood was obtained at enrolment (EDTA tube). After initial clinical testing (see below), the remaining plasma was processed and aliquoted for storage. A separate sample of whole blood was collected in EDTA tubes 24 h after admission, and aliquots of plasma and whole blood were prepared for storage. Plasma and whole blood were stored at − 80 °C within 8–24 h of collection and the samples were processed on subsequent dates for DNA extraction and other clinical laboratory tests. All of the laboratory testing for the present study was performed from the sample collected on enrolment with the exception of the CMV testing which was performed using the whole blood aliquot collected 24 h after admission. Biomarkers and *Pf*HRP-2 levels were tested in 2013, and CMV PCR and antibody testing in 2017.

Laboratory assessments

Thick and thin blood smears were assessed for *Plasmodium* species using microscopy with Giemsa staining to identify parasitaemia and calculate parasite density. Confirmatory testing was performed by a second laboratory technician to ensure accuracy. A complete blood count with differential, platelet count, glucose levels, and lactate levels were measured at Mulago Hospital. Additional testing was performed as clinically indicated. Testing for serum sodium, creatinine, and bilirubin levels was performed by the Makerere University Johns Hopkins University Core Lab. Plasma *P. falciparum* histidine-rich protein-2 was measured with the Malaria Antigen CELISA (Cellabs, Brookvale, NSW, Australia) and parasite biomass was calculated as described elsewhere [14].

CMV-specific testing was performed at the University of Minnesota Division of Pediatric Infectious Diseases Laboratory (CLIA ID: 24D1049829). Blood samples were purified from 100 µL of whole blood on a QIAcube (Qiagen, Hilden, Germany) using the manufacturer's specifications for the DNeasy Blood and Tissue kit. For CMV PCR, 10 µL of eluate was used in a reaction volume of 25 µL. Multiplex quantitative PCR with UL83 and NRAS primers and probes was performed as previously described [15] using the LightCycler 96 PCR System (Roche, Basel, Switzerland). The sensitivity of the assay was five or fewer copies of CMV per PCR. CMV seropositivity was assessed by Diamedix CMV Immunoglobulin G (IgG) Enzyme Immunoassay Test Kit (ERBA Diagnostics, Miami Lakes, FL) as previously described [16].

Multianalyte testing of plasma was performed using a Luminex MAP machine. Soluble markers of endothelial activation, including vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), E-selectin, and P-selectin, were measured using a cytometric bead assay (R&D Systems, Minneapolis, MN) with a Bio-Plex-200 system (Bio-Rad, Hercules, CA, USA) [17, 18]. C-reactive protein was measured using the Millipore CRP Luminex kit (Millipore-Sigma, Burlington, USA). Plasma angiopoietin-1 and angiopoietin-2 levels were quantified using a human angiopoietin-1 and angiopoietin-2 DuoSet ELISA (R&D Systems, Minneapolis, MN, USA). Asymmetric dimethylarginine (ADMA) levels in the plasma were quantified using ELISA (DLD Diagnostika GmbH, Hamburg, Germany) [17]. Arginine and superoxide dismutase levels were measured as previously described [19, 20].

AKI was defined according to the Kidney Disease: Improving Global Outcomes criteria with a single admission creatinine level and an estimated baseline creatinine level, as previously described [17]. CKD was defined as an estimated glomerular filtration rate < 90 mL/min/1.73 m^2 at the 12-month follow-up [21].

Neurocognitive outcomes were assessed at 1-week and 6-, 12-, and 24-month follow-up using the Mullen Scales of Early Learning, Color Object Association Test, and Early Childhood Vigilance Test in children to measure overall cognition, attention, and associative memory, respectively, in children aged < 5 years. For children aged > 5 years, the Kaufman Assessment Battery for Children (second edition) was used to measure overall cognition and working memory, and the Test of Variables of Attention was used to measure attention. Age-adjusted z-scores were calculated for all cognitive outcomes as previously described [13].

Statistical analysis

Data were analysed using Stata v18.0 (StataCorp, College Station, TX, USA). Demographic, clinical, and laboratory characteristics were compared between the CMV-DNAemic and CMV PCR-negative groups using Student's t-tests for continuous measures and Pearson's chi-squared or Fisher's exact test for categorical measures. Linear regression was used to assess the difference in the mean concentration of biomarkers of endothelial activation and nitrosative and oxidative stress in children with CMV DNAemia vs. CMV PCR-negative children. Results from simple linear regression were reported along with platelet-adjusted linear regression, where the log-transformed platelet count was included as a covariate in the models. For the Student's t-test and linear regression, non-Gaussian continuous measures were first log-transformed using the natural logarithm. Means and standard deviations were calculated from the log-transformed data, which were then back-transformed to the original scale for ease of interpretation in tables. As a result, the reported values represent geometric means and geometric standard deviations, which provide a more accurate measure of central tendency and variability for skewed data. The association between CMV viral load and platelet count was examined using the Spearman's correlation coefficient.

To measure the association between CMV viremia status and cognitive outcomes over a 24-month follow-up period, linear mixed-effects models were fit using within-subject correlations and subject-specific intercepts, including the four cognitive assessment time points, as categorical variables in the models. Additional covariates for the linear mixed-effects models were selected a priori and included age, socioeconomic status, maternal education, and height-for-age z-score at enrolment. Cognitive analyses were assessed by age group: (1) under 5 years at enrolment, (2) under 5 years at enrolment but turned 5 during the study (which includes children from the under 5 age group), and (3) always 5 years or older.

Results

Demographic characteristics and CMV status of the study population

Between 2008–2013, 269 children with CM were enrolled in this study. Twenty children died within the first 24 h of admission and did not have a sample available for CMV PCR testing, as the blood samples for CMV testing were collected at 24 h. An additional seven children did not have sufficient blood samples available for testing, leaving 242 children with CM who underwent CMV PCR testing.

The baseline characteristics of the study participants are presented in Table 1. The prevalence of CMV DNAemia after admission was 13.6% (N = 33). CMV IgG seropositivity was assessed in 104 randomly selected participants with CM, and 99% (N = 103) tested positive. The one child who was negative for CMV IgG did not develop DNAemia. Thus, CMV DNAemia in this cohort was not considered to represent primary infection.

Mortality and acute kidney injury

There was no significant difference in mortality between children who were CMV-DNAemic and PCR-negative (9.1% [N = 3] vs. 5.3% [N = 11], respectively; p = 0.42) (Table 1). Nevertheless, a substantial proportion of fatalities occurred within the initial 24 h period following admission, preceding the collection of whole blood for CMV analysis (N = 20), thereby limiting a comprehensive assessment of the impact of CMV DNAemia on mortality. There was an increased prevalence of AKI in CMV-DNAemic children compared to that in CMV PCR-negative children (59.4% [N = 19] vs. 38.6% [N = 78]; p = 0.03) (Table 1).

Clinical hematology indices and *P. falciparum* parasite burden

Haemoglobin and white blood cell counts did not differ according to DNAemia status, but C-reactive protein levels were elevated in the CMV PCR-negative group (p = 0.048) (Table 1). Unexpectedly, platelet counts were significantly higher in CMV-DNAemic versus PCR-negative participants (p = 0.02, Table 1). This finding persisted when controlling for *Pf*HRP2 levels (β 0.37 [0.08, 0.66], p = 0.014), but was no longer statistically significant when controlling for parasite density (β 0.26 [− 0.06, 0.57], p = 0.114). Among children with CMV-DNAemia, platelet levels did not correlate with CMV viral load (rho: 0.05, p = 0.79, N = 33). There was no significant association between parasite density and biomass, as calculated from *Pf*HRP2 levels, with the presence of CMV DNAemia (Table 1).

Endothelial cell and platelet activation

To further investigate the influence of CMV DNAemia on the pathogenesis of CM in relation to its association

Table 1 Demographic, clinical and laboratory characteristics of children with cerebral malaria, according to CMV PCR status

Characteristic	N	CMV DNAemia (n = 33)	N	CMV PCR-negative (n = 209)	P-value[a]
Age[b], years	33	3.3 (1.6)	209	3.6 (1.6)	0.315
Sex, male, n (%)	33	21 (63.6)	209	124 (59.3)	0.639
Weight for age z score baseline	33	− 1.0 (1.0)	208	− 1.0 (1.1)	0.993
Height for age z score baseline	33	− 1.3 (1.2)	209	− 0.98 (1.3)	0.175
HIV-positive, n (%)	33	2 (6.1)	207	3 (1.4)	0.141
Died, n (%)	33	3 (9.1)	209	11 (5.3)	0.415
Haemoglobin[b], g/dL	33	6.4 (1.6)	209	6.6 (1.4)	0.633
Severe anaemia[c], n (%)	33	10 (30.3)	209	44 (21.1)	0.226
WBC[b], cells/μL	33	11.3 (1.6)	206	9.6 (1.8)	0.135
Platelets[b], 10^3 platelets/μL	33	86.1 (2.6)	206	57.9 (2.3)	**0.012**
CRP[b], μg/mL	31	455 (2.7)	189	589 (1.8)	**0.048**
Acute kidney injury, n (%)	32	19 (59.4)	202	78 (38.6)	**0.027**
Parasite density[b], parasites/μL	29	30,495 (8.1)	207	43,012 (10.6)	0.458
PfHRP2[b], ng/mL	33	1909 (5.2)	209	2232 (3.7)	0.542
CMV viral load (copies/mL), median (IQR)	33	164 (107–301)		–	–

Mean (SD) reported, unless otherwise noted

CMV cytomegalovirus, *PCR* polymerase chain reaction, *WBC* white blood cell, *CRP* C-reactive protein, *PfHRP2 Plasmodium falciparum* histidine-rich protein II, *SD* standard deviation, *IQR* inter-quartile range

[a] Student's t-test used for continuous measures and Pearson's χ^2 or Fisher's exact test used for categorical measures

[b] Log-transformed, means (SD) are back-transformed in table, representing geometric means and geometric SDs

[c] Severe anaemia is defined as a haemoglobin level ≤ 5 g/dL

with AKI, an assessment of platelet and endothelial activation markers was conducted. The mean difference in log-transformed levels of factors was evaluated using linear regression (β coefficient providing mean difference) and adjusted for platelet count (aβ). In assessing markers of platelet activation, CMV DNAemia was associated with increases in plasma P-selectin (aβ, 0.39; $p<0.001$) and angiopoietin-1 (aβ, 0.59; $p=0.02$). Angiopoietin-1 was positively correlated with platelet counts (rho:0.244, $p=0.0004$), but its association with CMV-DNAemia persisted after controlling for platelet counts (Table 2). Soluble markers of endothelial activation (ICAM-1, VCAM-1, E-selectin, and angiopoietin-2) were not associated with CMV DNAemia (Table 2).

Oxidative and nitrosative stress

Markers of oxidative stress, including plasma levels and activity of superoxide dismutase, did not differ significantly according to the CMV DNAemia status. Plasma levels of ADMA, a marker of nitrosative stress, were elevated (β 0.19, $p=0.013$) in children with CMV-DNAemia (Table 3). Arginine and the arginine/ADMA ratio, were not different according to CMV DNAemia status.

Long-term outcomes: chronic kidney disease and neurocognitive impairment

There was no difference in the prevalence of CKD between the groups at the 12-month follow-up after admission for participants in whom follow-up data were available (CMV-DNAemic, 2/27 [7.4%] vs. CMV PCR-negative 7/159 [4.4%]). Similarly, neurocognitive outcomes for overall cognitive ability, attention, or memory over the 24-month follow-up period did not differ significantly by CMV DNAemia status for each age group assessed (Table 4).

Discussion

CMV DNAemia has been associated with mortality in adults with sepsis [8, 9]; however, limited studies on pediatric sepsis have been inconclusive [22, 23]. CMV DNAemia has not been previously evaluated in severe malaria. In this study of children with CM, CMV DNAemia was associated with AKI but not mortality during initial hospital admission. DNAemia was not associated with long-term neurocognitive impairment or CKD. CMV DNAemia was associated with relative elevations in platelet counts and markers of platelet activation, but

Table 2 Plasma concentrations of endothelial cell activation markers according to CMV PCR status

Biomarker	CMV DNAemia		CMV PCR-negative		Mean difference[a] β (95% CI)	P-value[a]	Platelet-adjusted mean difference[b,] aβ (95% CI)	aP-value[b]
	N	Mean (SD)	N	Mean (SD)				
ICAM-1, ng/mL	27	602.3 (5.5)	170	501.2 (3.6)	0.18 (– 0.37, 0.74)	0.512	0.38 (– 0.17, 0.93)	0.179
VCAM-1, ng/mL	27	3751 (1.7)	170	4320 (1.8)	– 0.14 (– 0.38, 0.10)	0.247	– 0.01 (– 0.23, 0.21)	0.929
E-selectin, ng/mL	27	185 (1.6)	170	179 (1.6)	0.03 (– 0.15, 0.21)	0.724	0.09 (– 0.09, 0.27)	0.349
P-selectin, ng/mL	27	69.9 (1.7)	162	50.5 (1.7)	0.32 (0.12, 0.53)	**0.002**	0.39 (0.18, 0.60)	**< 0.001**
Angiopoietin-1, ng/mL	27	5.0 (2.7)	181	2.4 (3.5)	0.75 (0.25, 1.25)	**0.004**	0.59 (0.11, 1.07)	**0.016**
Angiopoietin-2, ng/mL	27	1.9 (2.7)	181	1.7 (2.7)	0.11 (– 0.29, 0.51)	0.577	0.26 (– 0.14, 0.65)	0.203

All markers are log-transformed, means (SD) are back-transformed in table, representing geometric means and geometric SDs

CMV cytomegalovirus, *PCR* polymerase chain reaction, *ICAM-1* intercellular adhesion molecule 1, *VCAM-1* vascular cell adhesion protein 1

[a] Simple linear regression

[b] Linear regression adjusted for platelet count

Table 3 Plasma concentrations of markers of nitrosative and oxidative stress according to CMV PCR status

Biomarker	CMV DNAemia		CMV PCR-negative		Mean difference[a] β (95% CI)	P-value[a]
	N	Mean (SD)	N	Mean (SD)		
SOD-1 concentration, ng/mL	24	348 (2.2)	152	305 (2.0)	0.13 (– 0.18, 0.44)	0.403
SOD-1 activity (mUnits/ml)	24	206 (3.2)	158	306 (4.3)	– 0.40 (– 1.01, 0.22)	0.207
Arginine, µmol/dL	21	32.5 (1.6)	168	27.4 (1.6)	0.17 (– 0.05, 0.39)	0.122
ADMA, µmol/L	33	0.80 (1.4)	206	0.66 (1.5)	0.19 (0.04, 0.34)	**0.013**
Arginine/ADMA ratio	21	39.9 (1.5)	166	41.9 (1.7)	– 0.05 (– 0.28, 0.18)	0.671

All markers are log-transformed, means (SD) are back-transformed in table, representing geometric means and geometric SDs

CMV cytomegalovirus, *PCR* polymerase chain reaction, *SOD* superoxide dismutase, *ADMA* asymmetric dimethylarginine

[a] Simple linear regression

Table 4 Cognitive outcomes over 24 months in children with cerebral malaria, according to CMV PCR status

Group	Cognitive z-score	N (obs), N Total[a]	N (obs), N CMV DNAemia	Mean difference (95% CI), CMV DNAemia vs PCR-negative	P-value
Age < 5 at enrolment[b]	Cognitive ability	497, 169	68, 23	− 0.11 (− 0.61, 0.38)	0.65
	Attention	518, 169	73, 23	− 0.14 (− 0.51, 0.22)	0.44
	Associative memory	521, 170	74, 23	− 0.08 (− 0.28, 0.12)	0.42
Age < 5 at enrolment, but ≥ 5 at testing[b]	Cognitive ability	129, 93	14, 11	− 0.11 (− 0.60, 0.38)	0.67
	Attention	131, 93	14, 11	− 0.20 (− 0.90, 0.50)	0.57
	Working memory	131, 93	14, 11	0.15 (− 0.58, 0.88)	0.68
Age ≥ 5 at enrolment	Cognitive ability	208, 54	25, 7	− 0.52 (− 1.59, 0.54)	0.33
	Attention	207, 54	23, 7	0.15 (− 0.93, 1.24)	0.78
	Working memory	209, 54	25, 7	− 0.73 (− 1.83, 0.37)	0.19

Linear mixed-effects model, incorporating results of cognitive testing at all time points, adjusted for age, socioeconomic status, maternal education, and height-for-age z-score at enrolment. Mean difference in scores is the beta coefficient of this model. N (obs) = Number of observations, N = Number of subjects

CMV cytomegalovirus, *PCR* polymerase chain reaction

[a] This includes the total observations for all study participants with cerebral malaria (CMV DNAemia and CMV PCR-negative)

[b] All children who were < 5 years old at enrolment are included in the first row. The second row includes the subset of children from the first row who turned 5 years old at any point during the 24-month follow-up period

not with endothelial cell activation or oxidative stress. The association of CMV DNAemia with AKI and platelet activation requires confirmation in further studies. If confirmed, the mechanisms by which CMV DNAemia might contribute to AKI (or vice versa) in severe malaria should be further assessed.

AKI is a common complication of severe malaria and is associated with adverse outcomes [24]. Potential pathways contributing to AKI in malaria include kidney hypoperfusion due to dehydration and/or parasite sequestration, the release of nephrotoxic hemoproteins, endothelial activation, and tubulointerstitial inflammation [25]. In the present study, P-selectin was increased in children with CMV DNAemia. P-selectin is an endothelial cell adhesion marker stored within the membrane of Weibel-Palade bodies and platelet alpha granules, and is released in response to inflammatory stimuli, mediating leukocyte and platelet recruitment. P-selectin is upregulated in the cerebral vasculature in severe malaria [26], platelet-derived P-selectin has been implicated in the development of AKI in sepsis through neutrophil recruitment [27], and P-selectin levels have been independently associated with the presence and severity of AKI in severe human malaria [17]. CMV-mediated upregulation of P-selectin may be mediated through Toll-like receptor 2 (TLR2), which is expressed by a subpopulation of platelets [28]. CMV interacts with TLR2, and in the process this could trigger platelet activation and enhance P-selectin expression [29]. The activation of TLR2-positive platelets by CMV prompts the secretion of other inflammatory mediators, which in turn could augment the activation of platelets lacking TLR2 expression [29]. Thus, one potential pathway by which CMV may contribute to AKI could be via platelet activation and the consequent elevation of P-selectin levels.

Angiopoietin-1, which helps maintain endothelial quiescence, was also increased in children with CMV DNAemia relative to PCR-negative children, while there was no difference in angiopoietin-2, a marker of endothelial cell activation. Compared to the asymptomatic control group, angiopoietin-2 levels were elevated, and angiopoietin-1 levels were decreased in both the CMV-DNAemic and PCR-negative groups. Angiopoietin-2 is primarily stored in endothelial cell-derived Weibel-Palade bodies, and angiopoietin-1 is found in pericytes and platelets and stabilizes the endothelium. In severe and cerebral malaria, angiopoietin-1 is decreased compared to uncomplicated malaria or aparasitaemic controls, with a corresponding increase in angiopoietin-2 [17, 30], as is true in this CM cohort as a whole. Nonetheless, elevated angiopoietin-1 levels have been described in malaria and are attributed to platelet activation [31]. In the present study, the relative increase in angiopoietin-1 and lack of difference in angiopoietin-2 between the groups is consistent with elevated angiopoietin-1 being secondary to platelet number and in vivo platelet activation [32].

Children with CM and CMV DNAemia also show elevated levels of ADMA, an endogenous inhibitor of NO synthase [33]. In severe malaria, increased ADMA levels have been associated with decreased nitric oxide production, endothelial dysfunction, and increased mortality [33]. Elevated plasma ADMA levels are associated with AKI following cardiac catheterization [34] and may exacerbate AKI in murine models of renal ischemia–reperfusion injury [35]. Importantly, ADMA is partially excreted through the kidneys, and the observed elevation may be

attributable to the presence of AKI. When correcting for the presence of AKI, CMV DNAemia was no longer associated with plasma ADMA levels (αβ 0.14, p = 0.06). Additional studies evaluating urinary levels of ADMA would be helpful in clarifying the association between ADMA levels and CMV DNAemia.

Platelet levels are inversely proportional to malaria severity and contribute to the inflammatory response and endothelial dysfunction observed during infection, although they may also play a protective role [36]. Thrombocytopenia was present in most participants in this study, but there was an unexpected elevation of mean platelet levels in the CMV-DNAemic group compared to that in the PCR-negative group. This was unexpected since both malaria and CMV infections are associated with thrombocytopenia. In malaria, thrombocytopenia is primarily the result of increased platelet destruction [36]. In primary CMV infection, thrombocytopenia may result from either impaired platelet production or peripheral destruction through an immune-mediated process [37]; however, the impact of CMV reactivation on hematologic parameters in immunocompetent hosts is not well described. The mechanism behind the relative elevation in platelet levels in the CMV-DNAemic group is therefore uncertain. IL-6 has been implicated in both increased platelet production and CMV reactivation [7, 38, 39]; however, there was no significant difference in IL-6 levels based on the presence or absence of CMV in this cohort. Thrombopoietin levels were not measured, and it could not be determined whether there was increased production or decreased destruction during CMV reactivation. There was no association between platelet levels and *Pf*HRP2 in CMV-DNAemic versus CMV PCR-negative groups, but there was an inverse correlation between platelets levels and parasite density. This does not, however, explain why platelets were increased as higher platelet levels may be associated with lower parasite density through anti-parasitic effects [40]. Together, these results highlight the need for additional investigations to evaluate CMV-induced platelet activation, nitrosative stress, and AKI in children with CMV DNAemia, both in the context of cerebral malaria and independent of malarial infection.

The primary limitation of this study was the measurement of CMV DNAemia in samples obtained 24 h after admission. CMV-DNAemia could not be evaluated in children who died early during hospitalization and prior to the collection of whole blood for analysis; consequently, the data regarding the association between CMV DNAemia and mortality in children with CM is incomplete. Future studies investigating CMV reactivation in severe malaria should assess the viral load at enrolment and across multiple time points during hospitalization. The treatment of choice during the study period was quinine, an agent that has now been replaced by artesunate as the drug of choice for severe malaria; however, this pharmacologic difference should not have affected the primary findings of the study since both groups received the same intervention.

Conclusion

CMV DNAemia was present in 14% of children admitted with CM and was associated with AKI and elevation of plasma levels of the platelet activation markers P-selectin and angiopoietin-1, and the nitric oxide synthase inhibitor ADMA. CMV DNAemia 24 h after admission was not associated with mortality, long-term neurocognitive impairment, or CKD during the follow-up period. Future studies are needed to evaluate CMV DNAemia in children with severe malaria and to assess its relationship with disease severity, host response pathways, and clinical outcomes.

Abbreviations

ADMA	Asymmetric dimethylarginine
AKI	Acute kidney injury
CKD	Chronic kidney disease
CM	Cerebral malaria
CMV	Cytomegalovirus
IgG	Immunoglobulin G
ICAM-1	Intercellular adhesion molecule-1
TLR2	Toll-like receptor 2
VCAM-1	Vascular cell adhesion molecule-1

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Author contributions

CCJ and ROO designed and supervised the study. CCJ supervised general lab testing for the study. ALC supervised testing and analysis of acute kidney injury markers. MRS and NHA supervised testing of CMV DNAemia and seropositivity. PB supervised neuropsychological testing. JAM, AJW, and CCJ analyzed the results and wrote the first draft of the manuscript. CAB performed statistical analyses. All authors participated in the editing of the manuscript and approved the final version.

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Availability of data and materials

Data will be provided by the principal investigator upon reasonable request.

Declarations

Ethics approval and consent to participate

The Makerere University School of Medicine Research and Ethics Committee, and the University of Minnesota Institutional Review Board reviewed and approved the study. Informed consent forms were obtained from parents or guardians of study participants. The Ugandan National Council for Science and Technology provided regulatory review and approval.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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