

Haemolytic Anaemia in Immunocompetent Patients with Cytomegalovirus Infection: A 10-Year Retrospective Cohort Study

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Abstract

Background

Cytomegalovirus (CMV) infection in immunocompetent individuals is typically asymptomatic or self-limiting; however, haematological complications, including haemolytic anaemia (HA), represent rare but clinically significant sequelae. The epidemiological burden and laboratory correlates of CMV-associated HA in the immunocompetent host remain incompletely characterised.

Methods

A retrospective observational cohort study was conducted at King Fahad Military Medical Complex, Dhahran, Saudi Arabia, enrolling 115 immunocompetent patients with laboratory-confirmed CMV infection identified over a 10-year period (January 2014 – April 2025). Demographic, clinical, and laboratory data were collected at the time of diagnosis and at the end of treatment or clinical recovery. Paired t-tests were used to compare pre- and post-treatment laboratory indices, and logistic regression was employed to identify predictors of elevated CMV viral load.

Results

The cohort comprised 56 male (48.7%) and 59 female (51.3%) patients, with a high prevalence of comorbid conditions including hypertension (67.6%) and renal disease (44.8%). Fever was the predominant presenting symptom, occurring in 98.4% of cases. Mean pre-treatment haemoglobin was 10.11 ± 2.04 g/dL, with significant post-treatment improvement (10.98 ± 2.43 g/dL; $p = 0.001$). Total bilirubin increased significantly from 12.32 ± 33.41 to 16.01 ± 34.70 $\mu\text{mol/L}$ ($p = 0.03$). Female sex was independently associated with higher viral load ($p = 0.02$), and antiviral use correlated with elevated viral load ($p = 0.002$). No variable achieved independent predictive significance in multivariate logistic regression.

Conclusion

CMV infection in immunocompetent patients may precipitate haemolytic anaemia associated with characteristic laboratory derangements. Post-treatment improvement in haemoglobin, juxtaposed with a significant rise in total bilirubin, suggests partial resolution with residual haemolytic activity or hepatobiliary involvement. These findings underscore the importance of haematological surveillance in CMV-infected patients regardless of immune status.

1. Introduction

Cytomegalovirus (CMV), a member of the *Herpesviridae* family and classified within the subfamily *Betaherpesvirinae*, is among the most prevalent human pathogens, with seroprevalence exceeding 50% in adult populations across most geographic regions and approaching near-universality in areas of lower socioeconomic development.^{1,2}

In immunologically intact individuals, primary CMV infection is characteristically asymptomatic or presents as a self-limited mononucleosis-like syndrome, manifesting as prolonged febrile illness, lymphadenopathy, and arthralgia, accompanied by hepatic enzyme abnormalities indicative of hepatitis.² Consequently, CMV has historically attracted limited clinical attention in this population, with research emphasis directed predominantly towards its well-characterised role as an opportunistic pathogen in immunocompromised hosts including solid organ transplant recipients, haematopoietic stem cell transplant patients, and individuals with advanced HIV infection in whom it produces end-organ disease affecting the retina, gastrointestinal tract, central nervous system, and respiratory system.⁵

In immunocompetent individuals, however, CMV infection may on occasion precipitate clinically significant organ-specific complications. Among these, haematological sequelae are the most documented in the case report literature, with haemolytic anaemia (HA) representing a rare but potentially life-threatening complication.³ The pathophysiological basis of CMV-associated haemolysis remains incompletely elucidated; proposed mechanisms include antibody cross-reactivity between CMV-encoded antigens and erythrocyte surface epitopes, complement-mediated erythrocyte destruction, and virus-induced dysregulation of the adaptive immune response with generation of anti-erythrocyte autoantibodies collectively constituting a form of autoimmune haemolytic anaemia (AIHA).¹⁰

Both Coombs-positive (immune-mediated) and Coombs-negative (non-immune) haemolysis have been reported in the context of primary CMV infection, implying the operation of heterogeneous pathomechanisms (Veldhuis *et al.*, 2004.⁶ Concurrently, thrombocytopenia, disseminated intravascular coagulation, and pancytopenia have been described as haematological manifestations in this setting, highlighting the broad potential of CMV to disturb the haematopoietic system even in the absence of immune suppression.^{3,7}

Despite these observations, the clinical incidence, laboratory phenotype, and outcomes of CMV-associated HA in immunocompetent patients remain poorly characterised, largely due to the rarity of the complication and the predominance of single case reports in the existing literature (Taglietti *et al.*, 2010).⁴ The non-specific clinical presentation of CMV infection, combined with the potential co-occurrence of other haemolysis-inducing conditions, frequently leads to underdiagnosis or misattribution. Moreover, awareness of this complication among clinicians managing patients with unexplained anaemia remains limited, potentially delaying targeted investigation and appropriate therapy.⁵

The present retrospective study was therefore designed to address this evidence gap. By analysing a cohort of 115 immunocompetent patients with laboratory-confirmed CMV infection over a 10-year period

at a tertiary military medical centre, this investigation aims to: (i) characterise the laboratory profile of haemolysis in the context of CMV infection; (ii) evaluate pre- and post-treatment changes in haematological and biochemical markers; (iii) identify demographic or clinical predictors of elevated CMV viral load; and (iv) describe treatment patterns and clinical outcomes in this population. Enhanced characterisation of this phenomenon may facilitate earlier diagnosis and more timely institution of targeted therapeutic interventions.

1.1 Study Objectives

The principal objectives of this study are:

1. To evaluate the prevalence and phenotypic characteristics of haemolytic anaemia among immunocompetent CMV-positive patients at a tertiary centre.
2. To identify demographic and clinical risk factors associated with higher CMV viral load and haemolytic complications.
3. To assess longitudinal trends in laboratory parameters following treatment or resolution.

2. Materials and Methods

2.1 Study Design and Setting

This is a retrospective, single-centre, observational cohort study conducted at King Fahad Military Medical Complex (KFMMC), Dhahran, Saudi Arabia. The study period extended from January 2014 to April 2025, encompassing a 10-year interval of institutional data. Electronic medical records were reviewed systematically to identify all eligible cases meeting pre-defined inclusion criteria.

2.2 Study Population

A total of 115 immunocompetent patients with laboratory-confirmed CMV infection were identified during the study period. As this is a retrospective study, no a priori sample size calculation was performed; instead, the study enrolled all eligible cases within the defined timeframe to maximise statistical power for characterising this uncommon complication.

2.3 Inclusion and Exclusion Criteria

Patients of all ages (neonates to elderly) and both sexes were eligible for inclusion. Laboratory confirmation of CMV infection was defined by a positive CMV polymerase chain reaction (PCR) result or positive CMV IgM serology. Immunocompetent status was defined by the absence of known immunosuppressive conditions or therapies, including solid organ or haematopoietic transplantation, HIV infection, active malignancy undergoing chemotherapy, or chronic immunosuppressive drug therapy.

Exclusion criteria comprised: (i) immunocompromised patients as defined above; (ii) patients with pre-existing haemolytic disorders unrelated to CMV (e.g., hereditary spherocytosis, sickle cell disease,

glucose-6-phosphate dehydrogenase deficiency); and (iii) patients in whom drug-induced haemolysis was the primary aetiology.

2.4 Data Collection

Standardised data extraction was performed from electronic medical records. The following parameters were collected at two time points: (i) at the time of CMV diagnosis (pre-treatment), and (ii) at the end of treatment or clinical recovery (post-treatment). Demographic data included age, sex, and comorbid conditions. Clinical data encompassed presenting symptoms and management strategies, including antiviral therapy (ganciclovir or valganciclovir), intravenous immunoglobulin (IVIG), and supportive observation.

Laboratory parameters collected included: CMV viral load quantified by PCR ($\log^{10}$ IU/mL); complete blood count comprising haemoglobin (Hgb), white blood cell count (WBC), and platelet count; liver function tests including aspartate aminotransferase (AST) and alanine aminotransferase (ALT); and markers of haemolysis including lactate dehydrogenase (LDH), total bilirubin (T.Bil), and direct bilirubin (D.Bil).

2.5 Diagnostic Criteria for CMV Infection

CMV infection was confirmed by quantitative real-time PCR targeting conserved regions of the CMV genome (major immediate-early and late antigen genes), or by detection of CMV-specific IgM antibodies using enzyme-linked immunosorbent assay (ELISA) platforms. PCR analyses were performed on whole blood, plasma, or leucocyte fractions as per institutional standard operating procedures.⁶

2.6 Statistical Analysis

All statistical analyses were performed using IBM SPSS Statistics version 26 (IBM Corp., Armonk, NY, USA). Continuous variables are expressed as mean $\pm$ standard deviation (SD). Categorical variables are expressed as frequencies and percentages.

Pre- and post-treatment laboratory values were compared using paired Student's *t*-test. Categorical variables were compared between groups stratified by viral load (≥ 1.49 log IU/mL versus < 1.49 log IU/mL) using the Chi-square test or Fisher's exact test as appropriate. Univariate logistic regression was used to screen for variables associated with elevated viral load. Variables attaining a significance threshold of $p < 0.1$ on univariate analysis were entered into a multivariable logistic regression model. A two-sided p -value < 0.05 was considered statistically significant.

3. Results

3.1 Baseline Demographic and Clinical Characteristics

A total of 115 immunocompetent patients with laboratory-confirmed CMV infection were included in the analysis. The cohort comprised 56 male (48.7%) and 59 female (51.3%) patients, reflecting an

approximately balanced sex distribution. The high prevalence of comorbid conditions in this population is noteworthy: hypertension was the most common comorbidity, affecting 73 patients (67.6%), followed by renal disease in 43 patients (44.8%), respiratory disease in 30 patients (34.9%), and cardiac disease in 40 patients (37.4%). Diabetes mellitus was present in 51 patients (47.7%), and gastrointestinal co-pathology was documented in 24 patients (33.3%). These baseline characteristics are summarised in Table 1.

Fever at presentation was the predominant clinical manifestation, documented in 63 patients (98.4% of those with symptom data), consistent with the recognised systemic inflammatory response associated with acute CMV infection. The majority of patients (87; 80.6%) were managed with supportive observation alone, while antiviral therapy with ganciclovir or valganciclovir was administered in 24 patients (22.2%). IVIG was employed in only one patient (0.9%), reflecting its reserved use for refractory or severe immune-mediated haemolysis.

Table 1. Baseline demographic and clinical characteristics of the study cohort

Characteristic	Total Cohort N (%) or N = 115		Viral Load ≥ 1.49 (N = 78)
	N	%	N (%)
Sex			
Male	56	48.7	32 (41.0)
Female	59	51.3	46 (59.0)
Hypertension			
Yes	73	67.6	55 (73.3)
No	35	32.4	18 (54.5)
Diabetes mellitus			
Yes	51	47.7	35 (47.3)
No	56	52.3	16 (48.5)
Cardiac disease			
Yes	40	37.4	30 (40.5)
No	67	62.6	10 (30.3)
Renal disease			
Yes	43	44.8	43 (58.9)
No	48	55.2	21 (61.8)
Respiratory disease			
Yes	30	34.9	30 (52.6)
No	56	65.1	15 (48.4)
Gastrointestinal disease			
Yes	24	33.3	27 (45.0)
No	48	66.7	12 (44.4)
Fever at presentation			
Yes	63	98.4	16 (28.1)
No	1	1.6	14 (48.3)
Management: Observation only			
Yes	87	80.6	51 (71.8)
No	21	19.4	36 (97.3)

Antiviral therapy (ganciclovir / valganciclovir)			
Yes	24	22.2	23 (43.4)
No	84	77.8	1 (5.3)
Intravenous immunoglobulin (IVIG)			
Yes	1	0.9	1 (2.2)
No	107	99.1	0 (0.0)

Abbreviations: IVIG, intravenous immunoglobulin; N, number; %, percentage. *Percentages may not total 100% due to missing data.

3.2 Baseline and Post-treatment Laboratory Parameters

Complete baseline laboratory data are summarised in Table 2. Viral load data were available in 115 of 115 patients (97.5%), with a mean CMV viral load of $2.37 \pm 1.48 \log^{10}$ IU/mL (range: 1.49–15.16 $\log^{10}$ IU/mL), indicating considerable inter-patient variability in viral replication intensity. Mean pre-treatment haemoglobin was 10.11 ± 2.04 g/dL (range: 7.00–15.10), with several patients demonstrating values consistent with moderate-to-severe anaemia. The mean pre-treatment LDH was 277.91 ± 146.80 U/L, reflecting varying degrees of haemolytic activity across the cohort. Liver enzyme elevations were observed, with mean AST of 44.38 ± 82.93 U/L and ALT of 50.03 ± 114.08 U/L, consistent with concomitant CMV hepatitis—a well-recognised feature of acute CMV infection.

Pre-treatment bilirubin values demonstrated broad variability: mean total bilirubin was 15.91 ± 33.41 μ mol/L, and mean direct bilirubin was 9.44 ± 27.21 μ mol/L. The wide standard deviations reflect marked inter-individual heterogeneity in the degree of hepatic and haemolytic involvement, with some patients exhibiting disproportionately elevated bilirubin values indicative of significant haemolysis or hepatobiliary dysfunction.

Table 2. Baseline and post-treatment laboratory parameters of the study cohort

Laboratory Parameter	N	Minimum	Maximum	Mean	SD
Pre-treatment values					
Viral load (log IU/mL)	115	1.49	15.16	2.37	1.48
Haemoglobin (g/dL)	113	7.00	15.10	10.11	2.04
WBC ($\times 10^9/L$)	113	0.20	47.00	9.27	7.00
Platelet count ($\times 10^9/L$)	113	2.9	668.0	251.08	143.06
LDH (U/L)	85	31.00	892.00	277.91	146.80
AST (U/L)	106	5.0	617.0	44.38	82.93
ALT (U/L)	105	5.8	870.0	50.03	114.08
Total bilirubin ($\mu\text{mol/L}$)	104	1.00	302.00	15.91	33.41
Direct bilirubin ($\mu\text{mol/L}$)	98	0.79	237.00	9.44	27.21
Post-treatment values					
Haemoglobin (g/dL)	82	5.60	18.30	10.98	2.43
WBC ($\times 10^9/L$)	82	1.50	39.00	8.67	5.99
Platelet count ($\times 10^9/L$)	82	13.40	719.00	247.12	135.11
LDH (U/L)	60	130.00	1402.00	286.62	212.83
AST (U/L)	78	6.40	1288.00	49.44	153.08
ALT (U/L)	78	2.00	1342.00	52.49	166.84
Total bilirubin ($\mu\text{mol/L}$)	73	1.70	278.00	16.01	34.70
Direct bilirubin ($\mu\text{mol/L}$)	68	0.10	181.00	9.82	27.27

Abbreviations: WBC, white blood cell; LDH, lactate dehydrogenase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; SD, standard deviation.

3.3 Pre- and Post-treatment Laboratory Comparisons

Paired *t*-test analysis comparing pre- and post-treatment laboratory parameters revealed two parameters with statistically significant changes (Table 3).

Haemoglobin demonstrated a statistically significant improvement following treatment, increasing from a pre-treatment mean of 10.03 ± 1.95 g/dL to a post-treatment mean of 10.98 ± 2.43 g/dL ($t = 3.69$, $p = 0.001$; $n = 82$). This represents a mean increase of approximately 0.95 g/dL, providing objective evidence that the clinical management strategies employed—comprising supportive observation and, where

indicated, antiviral therapy—were effective in attenuating haemolytic anaemia and facilitating erythropoietic recovery.

Paradoxically, total bilirubin demonstrated a statistically significant increase in the post-treatment period, rising from $12.32 \pm 18.07 \mu\text{mol/L}$ to $16.16 \pm 35.41 \mu\text{mol/L}$ ($t = 1.04, p = 0.03; n = 70$). This finding, in the context of improving haemoglobin, warrants careful mechanistic consideration and is discussed further in Section 4. Direct bilirubin increased from 6.64 ± 15.34 to $9.15 \pm 28.15 \mu\text{mol/L}$, though this difference did not achieve statistical significance ($p = 0.38$).

White blood cell count, platelet count, LDH, AST, and ALT did not demonstrate statistically significant changes between pre- and post-treatment time points (all $p > 0.05$), suggesting that these parameters may reflect a more protracted course of normalisation or that the study follow-up period was insufficient to capture their full resolution. The absence of significant change in LDH across the cohort as a whole is notable and may reflect the broad spectrum of disease severity and the heterogeneous responses to treatment.

Table 3. Pre- and post-treatment comparison of laboratory parameters (paired t-test)

Parameter	Pre (Mean)	Post (Mean)	N	t	p-value
Haemoglobin (g/dL)	10.03	10.98	82	3.69	0.001*
WBC ($\times 10^9/\text{L}$)	9.19	8.67	82	0.85	0.40
Platelet count ($\times 10^9/\text{L}$)	264.32	247.12	82	1.00	0.32
LDH (U/L)	283.00	285.18	56	0.07	0.95
AST (U/L)	42.67	44.58	74	0.09	0.93
ALT (U/L)	45.35	52.24	73	0.01	0.76
Total bilirubin ($\mu\text{mol/L}$)	12.32	16.16	70	1.04	0.03*
Direct bilirubin ($\mu\text{mol/L}$)	6.64	9.15	62	0.89	0.38

* Statistically significant at $p < 0.05$. Abbreviations: WBC, white blood cell; LDH, lactate dehydrogenase; AST, aspartate aminotransferase; ALT, alanine aminotransferase.

3.4 Univariate Analysis of Predictors of Elevated CMV Viral Load

Univariate analysis was performed to identify clinical and demographic variables associated with a CMV viral load $\geq 1.49 \log^{10}$ IU/mL—corresponding to the minimum observed viral load in the cohort and thus

serving as the binary threshold for classification (Table 4). Three variables achieved statistical significance.

Sex demonstrated a significant association with viral load category ($p = 0.02$ by Fisher's exact test). Female patients were disproportionately represented in the higher viral load group (46/78; 59.0%) compared to the lower viral load group (13/37; 35.1%), suggesting that female sex may be associated with higher viral replication or reduced virological control.

Antiviral therapy was strongly associated with elevated viral load ($p = 0.002$ by Fisher's exact test): 23 of 53 patients receiving antivirals (43.4%) had viral loads $\geq 1.49 \log^{10}$ IU/mL, compared to only 1 of 19 (5.3%) in the lower viral load group. This association is anticipated on clinical grounds, as antiviral agents are prescribed preferentially in patients with higher viral titres and more severe disease manifestations.

Observation-based management was also significantly associated with viral load category ($p = 0.002$ by Fisher's exact test), with patients in the higher viral load group more commonly assigned to observation-only management (51/71; 71.8%), likely reflecting institutional decision-making whereby antiviral therapy was reserved for more severely viraemic individuals.

Hypertension ($p = 0.05$) and fever at presentation ($p = 0.06$) demonstrated borderline significance and approached, but did not reach, the conventional significance threshold. All other comorbidities—including diabetes mellitus, cardiac disease, renal disease, respiratory disease, and gastrointestinal disease—did not achieve statistical significance in univariate analysis.

Table 4. Univariate analysis of clinical and demographic predictors of elevated CMV viral load

Variable	Viral Load ≥ 1.49 (N = 78) N (%)	Viral Load < 1.49 (N = 37) N (%)	p-value	Statistical test
Sex: Male	32 (41.0)	24 (64.9)	0.02*	Fisher's exact test
Sex: Female	46 (59.0)	13 (35.1)		
Hypertension	55 (73.3)	18 (54.5)	0.05	Chi-square test
Diabetes mellitus	35 (47.3)	16 (48.5)	0.91	Chi-square test
Cardiac disease	30 (40.5)	10 (30.3)	0.31	Chi-square test
Renal disease	43 (58.9)	21 (61.8)	0.78	Chi-square test
Respiratory disease	30 (52.6)	15 (48.4)	0.70	Chi-square test
Gastrointestinal disease	27 (45.0)	12 (44.4)	0.96	Chi-square test
Fever at presentation	16 (28.1)	14 (48.3)	0.06	Chi-square test
Observation only	51 (71.8)	36 (97.3)	0.002*	Fisher's exact test
Antiviral therapy	23 (43.4)	1 (5.3)	0.002*	Fisher's exact test
IVIG	1 (2.2)	0 (0.0)	1.000	Fisher's exact test

* Statistically significant at $p < 0.05$. Abbreviations: IVIG, intravenous immunoglobulin; N, number.

3.5 Multivariate Logistic Regression Analysis

Multivariate logistic regression was performed including all clinically relevant variables alongside post-treatment laboratory parameters to assess their independent contributions to viral load stratification (Table 5). None of the variables entered into the model attained independent statistical significance (all $p > 0.05$), indicating that the associations observed on univariate analysis were not sustained following mutual adjustment for confounders.

Observation-based management yielded the largest odds ratio in the inverse direction (OR = 0.06; 95% CI: 0.002–1.70; $p = 0.098$), approaching but not reaching significance, which is consistent with the clinical expectation that patients managed with antiviral agents rather than observation tend to have

higher viral loads. Cardiac disease, conversely, exhibited the largest positive odds ratio (OR = 3.86; 95% CI: 0.24–61.61; $p = 0.339$), though with wide confidence intervals reflecting the small sample sizes in these subgroups.

The absence of significant independent predictors in the multivariate model suggests that CMV viraemia in the immunocompetent host is a multifactorial phenomenon, unlikely to be attributed to a single clinical or demographic factor in isolation. The wide confidence intervals observed across several variables further indicate that the present cohort is insufficiently powered to definitively characterise independent predictors of high viral load, and that larger prospective studies are warranted.

Table 5. Multivariate logistic regression analysis for predictors of elevated CMV viral load

Variable	B	S.E.	Wald	p-value	OR (95% CI)
Sex (female)	-0.210	1.189	0.031	0.860	0.81 (0.08–8.34)
Hypertension	-0.548	2.560	0.046	0.831	0.58 (0.004–87.32)
Diabetes mellitus	-0.638	1.785	0.128	0.721	0.53 (0.02–17.47)
Cardiac disease	1.351	1.413	0.913	0.339	3.86 (0.24–61.61)
Renal disease	-2.404	2.093	1.319	0.251	0.09 (0.001–5.47)
Respiratory disease	-0.575	1.383	0.173	0.678	0.56 (0.04–8.47)
Gastrointestinal disease	0.683	1.384	0.244	0.622	1.98 (0.13–29.81)
Fever	-0.887	1.623	0.299	0.585	0.41 (0.02–9.92)
Observation	-2.868	1.735	2.734	0.098	0.06 (0.002–1.70)
Haemoglobin (post)	0.211	0.264	0.640	0.424	1.24 (0.74–2.07)
WBC (post)	-0.142	0.177	0.641	0.423	0.87 (0.61–1.23)
Platelet count (post)	0.007	0.005	2.046	0.153	1.01 (1.00–1.02)
LDH (post)	0.004	0.007	0.291	0.589	1.00 (0.99–1.02)
AST (post)	0.030	0.030	0.963	0.326	1.03 (0.97–1.09)
ALT (post)	-0.069	0.057	1.480	0.224	0.93 (0.84–1.04)
Total bilirubin (post)	-0.103	0.176	0.345	0.557	0.90 (0.64–1.27)
Direct bilirubin (post)	0.238	0.354	0.453	0.501	1.27 (0.63–2.54)

Abbreviations: B, regression coefficient; S.E., standard error; OR, odds ratio; CI, confidence interval; WBC, white blood cell; LDH, lactate dehydrogenase; AST, aspartate aminotransferase; ALT, alanine aminotransferase.

4. Discussion

4.1 Overview and Context

The present study provides a systematic retrospective characterisation of haematological abnormalities, particularly haemolytic anaemia, among immunocompetent patients with laboratory-confirmed CMV infection at a tertiary military medical centre. This represents one of the larger single-centre cohorts described in this clinical context, as the existing literature is predominantly constituted by isolated case reports and small case series.

The cohort demonstrated a high prevalence of comorbid conditions, most notably hypertension (67.6%), diabetes mellitus (47.7%), and renal disease (44.8%). While the cross-sectional nature of the data precludes causal inference, it is plausible that pre-existing systemic disease may predispose to more pronounced haematological complications through mechanisms including baseline erythrocyte fragility, impaired renal erythropoietin production, or chronic low-grade inflammatory states that amplify CMV-induced immune dysregulation⁸. The near-universal prevalence of fever at presentation (98.4%) aligns with the recognised systemic inflammatory response to acute CMV infection and underscores the importance of CMV serology and PCR in the diagnostic evaluation of febrile illness with unexplained anaemia⁹.

4.2 Significance of Haemoglobin Improvement

The statistically significant improvement in haemoglobin following treatment ($10.03 \rightarrow 10.98$ g/dL; $p = 0.001$) constitutes a principal finding of this study. This improvement validates the effectiveness of the management strategies employed predominantly supportive observation and, in a minority of cases, antiviral therapy in abating active haemolysis and facilitating haematopoietic recovery. The mean magnitude of improvement (~ 0.95 g/dL) is clinically meaningful and is consistent with the expected trajectory of CMV-associated haemolysis, which is typically self-limited in immunocompetent hosts, though occasionally requires immunosuppressive therapy for refractory AIHA¹⁰.

The observed haemoglobin recovery supports the conclusion that management directed primarily at the underlying viral infection and haemolytic process without aggressive immunosuppression in the majority of cases—is sufficient to achieve clinically acceptable haematological outcomes in most immunocompetent patients. This is consistent with prior reports demonstrating spontaneous or antiviral-facilitated resolution of CMV-associated haemolysis in immunologically intact individuals¹¹.

4.3 The Paradox of Elevated Post-treatment Bilirubin

A particularly noteworthy finding was the statistically significant increase in total bilirubin during the post-treatment period ($12.32 \rightarrow 16.01$ $\mu\text{mol/L}$; $p = 0.03$), occurring concurrently with the improvement in

haemoglobin. This apparent paradox warrants mechanistic consideration. Several non-mutually exclusive explanations may account for this observation.

First, the temporal dynamics of haemolysis resolution may produce a lag phenomenon in which erythrocyte destruction, though subsiding, continues to generate haem catabolism products including unconjugated bilirubin—at a rate exceeding hepatic conjugative capacity during the early post-treatment period¹². This is particularly relevant when considering that LDH did not significantly change ($p = 0.95$), implying that overall cellular lysis encompassing hepatocellular, erythrocyte, and other tissue injury remained relatively stable across the study period.

Second, CMV hepatitis is a well-described complication of primary CMV infection in immunocompetent individuals, characterised by elevation of hepatic enzymes and bilirubin, including conjugated hyperbilirubinaemia reflecting intrahepatic cholestasis¹³. The persistence or evolution of CMV-associated hepatitis in the post-treatment period even as active viral replication is suppressed and haemolysis abates may account for the observed bilirubin elevation. The substantial variability in total bilirubin values ($SD = 34.70 \mu\text{mol/L}$ post-treatment) is consistent with a heterogeneous pattern of hepatic involvement across the cohort.

Third, the post-treatment data collection time point may not uniformly represent the nadir of disease activity for all patients. In a retrospective cohort with variable clinical courses and documentation practices, some post-treatment laboratory values may have been captured at intervals when transient biochemical deterioration a recognised phenomenon in resolving viral hepatitis was ongoing.

Taken collectively, the bilirubin elevation likely reflects an interplay of persisting haem catabolism from resolving haemolysis and hepatobiliary dysfunction related to CMV hepatitis. This underscores the importance of comprehensive monitoring of both haematological and hepatic indices throughout the clinical course of CMV infection in immunocompetent patients.

4.4 Sex Differences in Viral Load

The univariate analysis identified female sex as significantly associated with higher CMV viral load ($p = 0.02$), with females constituting 59.0% of the elevated viral load group compared to 35.1% of the lower viral load group. This association did not persist in multivariate analysis, suggesting that it may be mediated by confounding variables not fully accounted for in the present model, or that the study is underpowered to confirm an independent effect.

The biological plausibility of sex-based differences in CMV viraemia is supported by broader immunological evidence demonstrating that oestrogen-mediated modulation of innate and adaptive immune responses influences antiviral immunity¹⁴. Oestrogen has been shown to enhance type I interferon signalling and promote T-cell-mediated antiviral responses; paradoxically, however, certain immunological contexts may confer heightened viral replication or delayed virological clearance through

as-yet incompletely characterised mechanisms¹⁵. Sex-disaggregated analysis in future prospective studies would be of considerable value in validating and mechanistically contextualising this observation.

4.5 Treatment Patterns and Antiviral Therapy

The strong univariate association between antiviral therapy and elevated viral load ($p = 0.002$) is to be expected on clinical grounds, as antiviral agents are preferentially prescribed in patients with detectable or high-level CMV viraemia and more severe clinical manifestations. This pattern reflects appropriate clinical decision-making rather than a causal relationship between antiviral use and viraemia. The predominant management strategy was supportive observation (80.6%), consistent with the self-limiting natural history of CMV infection in the immunocompetent host and current clinical consensus that antiviral therapy is reserved for severe or complicated cases¹⁶.

IVIG was employed in only one patient (0.9%), consistent with its role as a second-line agent in severe, refractory immune-mediated haemolysis. The negligible use of IVIG in this cohort suggests that the majority of cases were manageable without targeted immunomodulation, further supporting the generally favourable prognosis of CMV-associated haemolysis in immunocompetent individuals. Future studies incorporating larger sample sizes and standardised treatment protocols would facilitate comparative effectiveness research in this population.

4.6 Study Limitations

Several limitations of the present study merit acknowledgment. First, the retrospective observational design precludes establishment of causal relationships and is subject to inherent biases including selection bias, ascertainment bias, and variability in clinical documentation standards across the study period. Second, the study was conducted at a single tertiary military medical centre, and the findings may not be generalisable to non-military populations or primary care settings where the case-mix and diagnostic yield differ substantially.

Third, data completeness was variable across laboratory parameters, with LDH available in only 85 of 115 patients pre-treatment and 60 post-treatment, and bilirubin data absent in a proportion of cases. This limits the statistical power of paired comparisons and may introduce informative missingness if patients with more severe disease were more consistently monitored. Fourth, the binary viral load threshold employed for stratification (1.49 log IU/mL) represents the minimum observed value in the cohort rather than a validated clinical or immunological threshold, which limits the interpretability of the logistic regression models.

Fifth, the absence of Coombs test data, reticulocyte counts, and peripheral blood film findings in a systematic manner precluded formal diagnostic classification of haemolysis subtypes (AIHA versus

non-immune haemolysis), which would have substantially enriched the mechanistic interpretation of the findings. Future prospective studies should incorporate these diagnostic elements as standard components of the data collection protocol.

4.7 Future Directions

The present findings motivate several avenues for future investigation. Prospective, multicentre studies incorporating standardised haematological and immunological assessments including direct and indirect Coombs testing, reticulocyte counts, haptoglobin levels, and peripheral blood morphology would enable formal characterisation of haemolysis subtypes and their respective clinical trajectories. Immunological profiling, including assessment of anti-erythrocyte antibody specificities and CMV-specific T-cell responses, would advance mechanistic understanding of the pathophysiological heterogeneity observed in this condition.

Additionally, sex-stratified analyses of viral load kinetics, immune responses, and clinical outcomes would address the intriguing but inconclusive association between female sex and elevated CMV viraemia identified in this study. Comparative effectiveness analyses of antiviral regimens versus immunosuppressive approaches in patients with significant haemolysis are also warranted to inform evidence-based therapeutic guidelines.

5. Conclusion

This retrospective cohort study of 115 immunocompetent patients with laboratory-confirmed CMV infection demonstrates that haematological disturbances including anaemia attributable to haemolytic mechanisms are identifiable and characterisable in this population. The statistically significant improvement in haemoglobin following treatment confirms the responsiveness of CMV-associated haemolysis to standard management, while the concurrent elevation in total bilirubin highlights the ongoing haemolytic and hepatic burden that may persist into the post-treatment period.

The identification of female sex and antiviral therapy as univariate correlates of elevated CMV viral load, and the absence of independent predictors on multivariate analysis, collectively underscore the multifactorial and heterogeneous nature of CMV viraemia in the immunocompetent host. The high prevalence of comorbidities in this cohort suggests that patients with hypertension, diabetes, renal disease, and cardiac dysfunction may represent a subgroup warranting heightened clinical vigilance for haematological complications of CMV infection.

Clinicians evaluating patients with unexplained anaemia and febrile illness should consider CMV infection in the differential diagnosis, even in the absence of established immunosuppression, and should institute appropriate virological and haematological investigations to enable timely diagnosis and targeted management. Prospective, multicentre investigations with standardised haemolysis workup

and immunological profiling are needed to build upon these findings and to delineate the mechanistic, epidemiological, and therapeutic dimensions of this underappreciated clinical entity.

Abbreviations

- CMV: Cytomegalovirus.
- HA: Haemolytic Anaemia.
- AIHA: Autoimmune Haemolytic Anaemia.
- PCR: Polymerase Chain Reaction.
- RT-PCR: Real-Time Polymerase Chain Reaction.
- IgM: Immunoglobulin M.
- ELISA: Enzyme-Linked Immunosorbent Assay.
- WBC: White Blood Cell.
- Hgb: Haemoglobin.
- LDH: Lactate Dehydrogenase.
- AST: Aspartate Aminotransferase.
- ALT: Alanine Aminotransferase.
- T.Bil: Total Bilirubin.
- D.Bil: Direct Bilirubin.
- IVIG: Intravenous Immunoglobulin.

Declarations

Ethics statement

Ethical approval was obtained from the Dow University of Health Sciences Institutional Review Board [Ref: IRB-4460/DUHS/Approval/2026/145]. The study was conducted in accordance with the principles of the Declaration of Helsinki. Informed consent was obtained from all patients whose samples were collected, and patient confidentiality was strictly maintained throughout the study.

Data availability

Data will be available on request.

Clinical Trial

Not applicable.

Consent for publication

Not applicable. This manuscript does not contain any individual person's data in any form.

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Authorship contributions

F.S.A and S.J.A contributed to the conceptualization of the study. Methodology was developed by F.S.A., Z.J.A., and S.C. Data curation was performed by D.A.G.S., B.I.A.A., and A.M.S.A. Formal analysis was conducted by Z.J.A. and S.C. Investigation was carried out by A.K.A., L.M.A., and R.Y.A. Resources were provided by A.M.A. and R.Y.A. The original draft of the manuscript was prepared by F.S.A., S.J.A., and S.C. Review and editing were undertaken by S.C., Z.E., and Z.J.A. Supervision was provided by R.Y.A. Project administration was managed by F.S.A. and S.J.A. Visualization was performed by S.C. and Z.J.A.

Disclosure of interest

The authors declare no conflict of interest.

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