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Absence of Detectable Cytomegalovirus DNAemia by Conventional Qualitative PCR in Non-Transplant Critically Ill Renal Failure Patients Despite High Transfusion Exposure: A Cross-Sectional Molecular Surveillance Study and Diagnostic Threshold Analysis

Komal Khalid[1], **Nadeem Sharif**[2]

[1] Department of Molecular Biology and Medical Laboratory Technology, Gulab Devi Postgraduate Medical Institute (PGMI) / University of Health Sciences, Lahore, Pakistan

[2] Department of Pathology, Isfandyar Bukhari District Hospital, Attock, Punjab, Pakistan, **Corresponding Author:** Email: nadeem.sharif@uo.edu.pk, ORCID: 0009-0002-8360-6296

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Abstract

Human cytomegalovirus (HCMV) reactivation causes substantial morbidity in classically immunosuppressed transplant recipients and mechanically ventilated septic intensive care unit (ICU) patients. Patients with acute and chronic end-organ renal failure undergoing maintenance hemodialysis experience uremia-associated adaptive immune dysfunction alongside frequent exposure to non-leukoreduced blood transfusions. However, whether uremic dysregulation and transfusion burden precipitate clinically detectable whole-blood HCMV DNAemia in non-transplant hospitalized renal failure patients—and whether conventional qualitative end-point polymerase chain reaction (PCR) provides adequate diagnostic sensitivity—remains poorly defined in South Asian settings. We conducted a STROBE-compliant cross-sectional molecular surveillance study over six months among 33 critically ill adults with acute or chronic renal failure managed at tertiary care units in Lahore, Pakistan. Standardized clinical, comorbidity, transfusion, hemodialysis vintage, hematological, and renal biochemical parameters were recorded. Genomic DNA was purified from 300 µL EDTA-anticoagulated whole blood and verified for nucleic acid integrity and absence of polymerase inhibitors via human *ACTB* (beta-actin, 110 bp) amplification. Qualitative HCMV amplification targeting a 222 bp viral sequence was performed alongside positive and negative controls. Exact Clopper–Pearson, Wilson score, and one-sided 95% upper prevalence bounds, exact binomial benchmark tests, Bayesian Beta-Binomial posterior updating, and stoichiometric limit-of-detection (LOD) probit analyses were conducted. Among 33 enrolled patients (mean age 45.24 ± 17.01 years; 66.7% male), 27 (81.8%) had chronic renal failure and 6 (18.2%) had acute renal failure. Thirty-one patients (93.9%) were receiving maintenance hemodialysis (median vintage 24.0 months, interquartile range 12.0–36.0 months), 31 (93.9%) had systemic hypertension (15.2% with comorbid diabetes mellitus), and 26 (78.8%) had received allogeneic blood transfusions. Laboratory evaluation confirmed severe azotemia (mean serum creatinine 6.38 ± 3.86 mg/dL) and moderate renal anemia (hemoglobin 9.49 ± 1.45 g/dL) with preserved total leukocyte counts (7.16 ± 2.44 × 10^3/µL). All 33 specimens (100%) yielded robust 110 bp *ACTB* internal control bands, whereas 0/33 (0.0%; two-sided exact 95% CI: 0.00%–10.58%; one-sided 95% upper bound: 8.68%) tested positive for HCMV DNA. Exact binomial tests rejected equivalence with published septic/ventilated ICU reactivation rates of 16.9%–32.5% ($P = 0.004$ to $P = 4.9 \times 10^{-6}$) while remaining statistically concordant with international hemodialysis PCR cohorts (0.0%–2.7%; $P = 0.41$–1.00). Bayesian updating under hemodialysis-informed priors yielded a posterior median prevalence of 1.78% (95% credible interval: 0.58%–4.01%; 99.5% probability that true high-level viremia is <5%). Non-transplant hospitalized patients with severe acute or chronic renal failure do not exhibit prevalent high-burden whole-blood

HCMV DNAemia despite prolonged hemodialysis vintage and extensive non-leukoreduced transfusion exposure. Routine cross-sectional screening with single-round qualitative gel PCR (analytical LOD ~500–830 copies/mL) has low diagnostic yield in non-immunosuppressed renal cohorts; diagnostic resources in low- and middle-income settings should instead prioritize WHO-calibrated quantitative real-time PCR for patients developing refractory sepsis, prolonged mechanical ventilation, or pre-transplant risk stratification.

Keywords: Human Cytomegalovirus; DNAemia; Chronic kidney disease; Hemodialysis; Blood Transfusion; Polymerase Chain Reaction; Analytical Sensitivity; Zero-Event Binomial Inference; Pakistan

Introduction

Human cytomegalovirus (HCMV; *Human betaherpesvirus 5*, family *Orthoherpesviridae*, subfamily *Betaherpesvirinae*) possesses the largest and most genetically complex linear double-stranded DNA genome (~235 kilobases encoding more than 225 open reading frames) among human herpesviruses [1,2]. Following primary mucosal or parenteral acquisition—which typically occurs during early childhood and is clinically unapparent or manifests as a self-limited mononucleosis-like syndrome in immunocompetent hosts—HCMV establishes lifelong episomal latency within bone marrow CD34+ hematopoietic progenitor cells, peripheral blood CD14+ monocytes, and tissue-resident vascular endothelial cells [2–4]. Global seroepidemiological syntheses demonstrate a pronounced socioeconomic gradient in latent HCMV carriage: adult immunoglobulin G (IgG) seroprevalence averages 40%–65% across Western Europe and North America but exceeds 85%–98% across South Asia, the Middle East, Latin America, and sub-Saharan Africa [5–8]. Within Pakistan, regional blood bank surveys in Lahore and Abbottabad have documented anti-HCMV IgG seropositivity in 90.2% to 97.8% of healthy adult blood donors [7,8], establishing an almost universal reservoir of latent infection capable of endogenous reactivation during states of host immune compromise.

Historically, clinically consequential HCMV end-organ disease—encompassing interstitial pneumonitis, gastrointestinal ulceration, hepatitis, myocarditis, and necrotizing retinitis—was considered restricted to profoundly immunosuppressed populations, including solid-organ and allogeneic hematopoietic stem cell transplant recipients, neonates with congenital infection, and individuals with advanced human immunodeficiency virus (HIV) infection prior to the widespread introduction of antiretroviral therapy [2,9–11]. Over the past two decades, however, prospective molecular surveillance has reshaped this paradigm by demonstrating that 15% to 40% of apparently immunocompetent adults admitted to medical, surgical, burn, and trauma intensive care units (ICUs) experience systemic or pulmonary HCMV reactivation, which correlates with prolonged mechanical ventilation, nosocomial bacterial or fungal superinfection, acute kidney injury, and roughly two- to three-fold higher crude mortality [12–17]. Mechanistically, inflammatory stressors such as bacterial lipopolysaccharide, tumor necrosis factor-alpha (TNF-alpha), and catecholamine surges drive chromatin remodeling of the viral major immediate-early promoter (MIEP) as latent monocytes differentiate into tissue macrophages, while sepsis-induced T-lymphocyte exhaustion impairs cytotoxic CD8+ and CD4+ immune surveillance [4,18–20].

Patients with severe acute kidney injury and end-stage chronic renal failure undergoing maintenance hemodialysis occupy a distinct, clinically ambiguous immunological niche between immunocompetent outpatients and classically immunosuppressed transplant recipients [21,22]. Uremic toxin retention, chronic dialyzer membrane bio-incompatibility, oxidative stress, and metabolic acidosis induce profound functional defects across both innate and adaptive immunity—characterized by impaired monocyte costimulatory signaling, premature thymic involution, contraction of naive T-cell repertoires, and expansion of terminally differentiated, senescent CD4+CD28- and CD8+CD28- T-cell populations [21,22]. Concurrently, erythropoietin-deficient renal anemia frequently necessitates repeated allogeneic packed red blood cell transfusions in resource-constrained nephrology centers where universal pre-storage leukoreduction and CMV-seronegative blood inventories are unavailable [7,23,24]. Because latent

HCMV resides within donor peripheral blood leukocytes, frequent transfusion theoretically exposes uremic recipients to both transfusion-transmitted primary infection and allogeneic superinfection or cytokine-mediated reactivation of endogenous virus [6,23,24].

Despite this convergence of uremic immunosenescence and high transfusion burden, published estimates of active HCMV infection in hemodialysis and non-transplant renal failure cohorts remain markedly discordant [25–28]. Serological surveys relying on anti-HCMV IgM antibodies report apparent active infection rates of 5% to 15% among hemodialysis patients [25,27], yet molecular investigations utilizing quantitative nucleic acid amplification have challenged the specificity of IgM serology in uremia, reporting zero or near-zero systemic viremia in stable maintenance dialysis cohorts [26,28]. Furthermore, in low- and middle-income countries (LMICs), hospital molecular laboratories frequently rely on conventional qualitative end-point gel PCR rather than serial World Health Organization (WHO)-calibrated real-time quantitative PCR (qPCR) [29,30], and small single-center studies reporting zero positive events often misinterpret a zero numerator as definitive proof of zero viral risk without accounting for binomial confidence bounds or assay limit-of-detection (LOD) stoichiometry [31,32].

To address these clinical and methodological gaps, we conducted a six-month cross-sectional molecular surveillance study evaluating whole-blood HCMV DNAemia using internal-control–validated qualitative PCR in hospitalized patients with acute and chronic end-organ renal failure in Lahore, Pakistan. Beyond characterizing the clinical, hematological, biochemical, dialysis-vintage, and transfusion profiles of this high-exposure South Asian cohort, we apply exact binomial confidence estimation, post-hoc statistical power benchmarking, Bayesian Beta-Binomial posterior updating, and stoichiometric LOD modeling to rigorously define the upper bounds of high-level HCMV viremia and establish evidence-based diagnostic recommendations for resource-limited renal and critical care units.

Materials and Methods

Study Design, Setting, and Reporting Standards

An observational cross-sectional molecular diagnostic surveillance study was conducted over a six-month period (July through December) at the Molecular Biology Laboratory of Gulab Devi Chest Hospital / Postgraduate Medical Institute (PGMI) in collaboration with tertiary renal and critical care services at Gulab Devi Hospital and Lahore General Hospital, Lahore, Pakistan. Study reporting adheres to the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines for cross-sectional investigations [33] and incorporates relevant analytical transparency elements from the Minimum Information for Publication of Quantitative/Qualitative Molecular Experiments (MIQE) framework [34].

Participant Eligibility and Clinical Data Collection

Adult inpatients (aged >=18 years) consecutively evaluated during the study period were eligible for enrollment if they met the following inclusion criteria: (i) documented critical illness with acute renal failure (acute kidney injury) or chronic end-organ renal failure (chronic kidney disease stage 5 / end-stage kidney disease) requiring inpatient medical stabilization or renal replacement therapy; (ii) hospitalization duration of >=5 days with expected clinical survival >72 hours from enrollment; (iii) presence of clinical features compatible with systemic inflammatory stress or potential occult viral syndrome (such as unexplained prolonged fever, clinical sepsis, or progressive organ dysfunction) and/or documented inpatient or recent allogeneic blood transfusion exposure; and (iv) provision of written informed consent by the patient or legally authorized next of kin. Patients receiving pharmacological immunosuppression for prior solid-organ or hematopoietic stem cell transplantation, cytotoxic chemotherapy, or known HIV/AIDS, as well as those declining consent or hospitalized <5 days, were excluded.

A structured clinical case-report instrument was completed for every participant at specimen collection, capturing demographic variables (age in years, biological sex), renal failure classification (acute vs. chronic renal failure), maintenance hemodialysis status and cumulative dialysis vintage (months/years), lifetime and recent history of allogeneic blood product transfusion, and physician-documented

comorbidities (systemic arterial hypertension, diabetes mellitus, and microbiologically confirmed bacterial co-infections including methicillin-resistant *Staphylococcus aureus* [MRSA]).

Hematological and Renal Biochemical Assays

Venous peripheral blood samples were collected aseptically by trained phlebotomists into dipotassium ethylenediaminetetraacetic acid (K_2-EDTA) vacutainer tubes for hematological and molecular analyses and plain serum-separator tubes for renal biochemistry. Automated complete blood counts—including hemoglobin concentration (g/dL), total leukocyte count ($\times 10^3/\mu L$), differential neutrophil and lymphocyte percentages (%), and platelet count ($\times 10^3/\mu L$)—were performed immediately upon collection. Serum urea (mg/dL) and serum creatinine (mg/dL) concentrations were quantified on automated clinical chemistry analyzers using standardized enzymatic urease and kinetic Jaffe colorimetric methods, respectively. EDTA whole-blood aliquots designated for viral nucleic acid extraction were maintained at 4°C under cold-chain conditions prior to processing.

Genomic DNA Purification and Internal Amplification Control (ACTB)

Total genomic and viral DNA was extracted from 300 µL of homogenized EDTA whole blood using the Thermo Scientific Genomic DNA Purification Kit (Catalog No. K0512; Thermo Fisher Scientific, Waltham, MA, USA) strictly in accordance with the manufacturer's protocol. Briefly, 300 µL of whole blood was combined with 400 µL of cell lysis solution and incubated at 65°C for 30 minutes. Immediately thereafter, 600 µL of molecular-grade chloroform was added, gently emulsified by 3–5 tube inversions, and phase-separated by centrifugation at 10,000 rpm for 2 minutes. Working precipitation solution was freshly prepared by diluting 80 µL of 10× concentrated precipitation buffer with 720 µL of sterile deionized water. The upper aqueous DNA-containing phase was transferred to a sterile microcentrifuge tube, mixed gently with 800 µL of working precipitation solution for 1–2 minutes at room temperature, and centrifuged at 10,000 rpm for 2 minutes. Following complete aspiration of the supernatant, the nucleic acid pellet was dissolved in 100 µL of NaCl solution by gentle vortexing until fully solubilized, precipitated with 300 µL of ice-cold isopropanol at -20°C for 20 minutes, and pelleted at 10,000 rpm for 3–4 minutes. After complete removal of residual isopropanol, the purified DNA pellet was resuspended in 50 µL of sterile nuclease-free deionized water, yielding a 6-fold volumetric concentration factor (1 µL eluate corresponding to 6 µL of starting whole blood).

To verify genomic DNA recovery, confirm structural template integrity, and rule out carryover of heme, EDTA, or intracellular Taq DNA polymerase inhibitors in every clinical extract, an endogenous human housekeeping control PCR targeting a 110 bp segment of the beta-actin (*ACTB*) gene was performed prior to viral scoring. Reactions were assembled on ice in sterile 0.2 mL thin-walled PCR tubes in a final volume of 20 µL containing 1× Taq reaction buffer, 1.5 mM $MgCl_2$, 250 µM of each deoxynucleoside triphosphate (dNTP), forward primer (5'-GCAACCTTGGGAACAATACG-3'; 20-mer), reverse primer (5'-CCACGTTGTCCATGAAGAGG-3'; 20-mer), 1 U recombinant Taq DNA polymerase, and 2 µL (~1–10 ng) extracted template DNA, alongside positive human genomic DNA and no-template negative water controls. Thermal cycling comprised initial denaturation at 95°C for 5 minutes; 35 cycles of denaturation at 94°C for 45 seconds, primer annealing at 54°C for 45 seconds, and extension at 72°C for 45 seconds; and a final extension at 72°C for 5 minutes. Ten microliters of amplified product mixed with tracking buffer was resolved by electrophoresis through a 2% (w/v) agarose gel stained with ethidium bromide and inspected under ultraviolet (UV) transillumination against a molecular-weight DNA ladder; only extracts exhibiting a crisp 110 bp *ACTB* amplicon were deemed analytically valid.

Qualitative HCMV DNA Polymerase Chain Reaction

Qualitative detection of HCMV genomic DNA was performed using a commercial *in vitro* diagnostic qualitative end-point PCR kit (CMV PCR Detection Kit, Catalog No. PR7836C; CinnaGen Inc., Tehran, Iran) targeting a conserved region of the HCMV genome generating a 222 bp diagnostic amplicon. Reagents were thawed on ice and pulse-centrifuged before opening. Each 25 µL amplification reaction

contained 20 µL of 1× pre-aliquoted CMV PCR Master Mix (containing target-specific primers, dNTPs, and optimized reaction buffer with internal dye), 0.3 µL of Taq DNA polymerase, and 5.0 µL of extracted template DNA (representing 30 µL of original EDTA whole-blood equivalent), overlaid with 20–25 µL of sterile mineral oil to prevent evaporative concentration shifts. Every analytical batch included a kit-supplied cloned HCMV positive control template and a nuclease-free water negative contamination control.

Amplification was executed in a preheated thermal cycler using the manufacturer-validated two-stage touch-down/step-down profile: 1 initial cycle of denaturation at 95°C for 180 seconds, annealing at 62°C for 40 seconds, and extension at 72°C for 40 seconds; followed by 35 cycles of denaturation at 93°C for 40 seconds, annealing at 61°C for 40 seconds, and extension at 72°C for 40 seconds. Following amplification, 10 µL of PCR product was loaded directly onto a 2% agarose gel containing ethidium bromide and electrophoresed alongside a DNA size marker and kit controls. Specimens producing a distinct 222 bp UV-fluorescent band co-migrating with the positive control in the absence of amplification in the negative control were defined as HCMV DNAemia–positive.

Statistical Analysis, Zero-Event Binomial Inference, and Bayesian / Analytical Modeling

Baseline descriptive statistics were initially compiled in SPSS version 16.0 and extended using exact analytical routines in Python 3.12 (NumPy 2.x and SciPy `stats`). Continuous clinical and laboratory variables are summarized as mean ± standard deviation (SD) and range (minimum–maximum), while skewed ordinal exposures (hemodialysis vintage) are additionally summarized as median and interquartile range (IQR). Categorical variables are reported as counts, percentages, and exact two-sided 95% Clopper–Pearson binomial confidence intervals (CIs) [31].

Because conventional asymptotic Wald intervals collapse degenerately to `[0%, 0%]` when zero events (`k = 0`) are observed in a finite sample (`n`), four complementary exact and small-sample frequentist bounds were computed across the full cohort (`N = 33`) and pre-specified clinical risk strata (maintenance hemodialysis, chronic renal failure, blood-transfused subgroup, prolonged dialysis vintage >=12 and >=24 months, sex, and acute renal failure): (i) two-sided 95% exact Clopper–Pearson upper limits (`1 - 0.025^(1/n)`) [31]; (ii) Wilson score 95% upper bounds; (iii) exact one-sided 95% upper confidence limits (`1 - 0.05^(1/n)`); and (iv) Hanley and Lippman-Hand's rule-of-three approximation (`3/n`) [32]. Post-hoc statistical detection power (`1 - (1 - p)^33`) was modeled continuously across hypothesized true prevalence values `p`, and exact two-sided and one-sided binomial tests were performed against published HCMV prevalence benchmarks from hemodialysis PCR cohorts [26,28] and non-immunosuppressed ICU cohorts [12–14,16].

To integrate external epidemiological knowledge formally, Bayesian conjugate Beta-Binomial updating (`Posterior = Beta(alpha_0 + k, beta_0 + n - k)`) was conducted under four prior specifications: (i) an uninformative Jeffreys prior `Beta(0.5, 0.5)`; (ii) a flat uniform prior `Beta(1.0, 1.0)`; (iii) an informative hemodialysis molecular prior `Beta(4.5, 197.5)` derived from pooled international hemodialysis PCR cohorts (`0/52` in Pliquett et al. [26] and `4/149` in Kelishadi et al. [28]); and (iv) a weakly informative ICU reactivation prior `Beta(2.7, 7.3)` centered at the 27.0% meta-analytic ICU prevalence reported by Li et al. [16]. Finally, stoichiometric probit detection curves were constructed to compare the 95% analytical limit of detection (`LOD95`) of conventional single-round gel PCR (30 µL whole-blood equivalent/reaction) against WHO-standardized real-time qPCR [29,30] and pp65 leukocyte antigenemia.

Results

Demographic, Renal Disease, Dialysis Vintage, and Transfusion Characteristics

Thirty-three hospitalized adults meeting all clinical inclusion criteria were enrolled and evaluated over the six-month surveillance period (**Table 1**, **Figure 1A–B**). Patient age spanned six decades from 21 to 80 years, with a cohort mean of 45.24 ± 17.01 years. Males outnumbered females in an exact 2:1 ratio (22 males [66.67%; 95% CI: 48.17%–82.04%] vs. 11 females [33.33%; 95% CI: 17.96%–51.83%]). Chronic renal failure (end-stage kidney disease / CKD stage 5) constituted the predominant underlying end-organ

pathology, accounting for 27/33 patients (81.82%; 95% CI: 64.54%–93.02%), whereas 6/33 patients (18.18%; 95% CI: 6.98%–35.46%) presented with severe acute renal failure.

Thirty-one of the 33 patients (93.94%; 95% CI: 79.77%–99.26%) were receiving maintenance intermittent hemodialysis at enrollment, while 2 patients (6.06%) with acute renal decompensation had not yet initiated chronic dialysis (**Table 1**, **Figure 1B**). Across all 33 patients, cumulative hemodialysis duration averaged 21.33 ± 14.51 months (median 24.0 months; IQR 6.0–36.0 months; range 0–48 months). Restricted to the 31 dialyzed patients, mean dialysis vintage was 22.71 ± 13.87 months (median 24.0 months; IQR 12.0–36.0 months): 7 patients (21.21% of the total cohort; 22.58% of dialyzed patients) had been on hemodialysis for 3–6 months, 5 (15.15%) for 12 months, 8 (24.24%) for 24 months, 9 (27.27%) for 36 months, and 2 (6.06%) for 48 months. Notably, 24/33 patients (72.73%) had >=12 months of cumulative extracorporeal dialysis exposure.

Cardiovascular and metabolic comorbidities were highly prevalent (**Table 1**, **Figure 1A**). Systemic arterial hypertension was documented in 31/33 patients (93.94%; 95% CI: 79.77%–99.26%), comprising 26 patients (78.79%) with isolated hypertension and 5 patients (15.15%) with concomitant hypertension and diabetes mellitus. One patient (3.03%) had microbiologically documented MRSA infection, and 1 patient (3.03%) had no secondary systemic comorbidity recorded beyond primary renal failure. Crucially, 26/33 patients (78.79%; 95% CI: 61.09%–91.02%) had a documented history of at least one allogeneic blood transfusion during their disease course, whereas only 7 patients (21.21%) were transfusion-naive.

Table 1. Baseline demographic, renal, comorbidity, transfusion, and hemodialysis vintage characteristics of the end-organ renal failure cohort (*N* = 33).

Clinical / Demographic Parameter	Category or Metric	Value (n / N or Stat)	Percentage or Range	Exact 95% CI (%)
Age (years)	Mean ± SD (Min–Max)	45.24 ± 17.01	21.0 – 80.0	—
Biological Sex	Male	22 / 33	66.67%	48.17 – 82.04
	Female	11 / 33	33.33%	17.96 – 51.83
Renal Failure Phenotype	Chronic renal failure (CKD stage 5)	27 / 33	81.82%	64.54 – 93.02
	Acute renal failure (AKI)	6 / 33	18.18%	6.98 – 35.46
Blood Transfusion Exposure	History of >=1 blood transfusion	26 / 33	78.79%	61.09 – 91.02
	No prior transfusion	7 / 33	21.21%	8.98 – 38.91
Comorbidity Profile	Hypertension (any, isolated or combined)	31 / 33	93.94%	79.77 – 99.26
	Hypertension alone	26 / 33	78.79%	61.09 – 91.02
	Hypertension + Diabetes mellitus	5 / 33	15.15%	5.11 – 31.90

Clinical / Demographic Parameter	Category or Metric	Value (n / N or Stat)	Percentage or Range	Exact 95% CI (%)
	MRSA co-infection	1 / 33	3.03%	0.08 – 15.76
	No secondary comorbidity recorded	1 / 33	3.03%	0.08 – 15.76
Hemodialysis Status	Active maintenance hemodialysis	31 / 33	93.94%	79.77 – 99.26
	Non-dialyzed at sampling (0 months)	2 / 33	6.06%	0.74 – 20.23
Dialysis Vintage (All, *N* = 33)	Mean ± SD / Median (IQR), months	21.33 ± 14.51	24.0 (6.0 – 36.0)	—
Dialysis Vintage (HD, *n* = 31)	Mean ± SD / Median (IQR), months	22.71 ± 13.87	24.0 (12.0 – 36.0)	—
Dialysis Duration Strata	Early HD: 3–6 months (3, 4, 5, 6 mo)	7 / 33	21.21% (2, 1, 2, 2)	8.98 – 38.91
	Intermediate HD: 12 months (1 year)	5 / 33	15.15%	5.11 – 31.90
	Established HD: 24 months (2 years)	8 / 33	24.24%	11.09 – 42.26
	Prolonged HD: 36–48 months (3–4 years)	11 / 33	33.33% (9, 2)	17.96 – 51.83

Abbreviations: AKI, acute kidney injury; CI, exact two-sided Clopper–Pearson confidence interval; CKD, chronic kidney disease; HD, hemodialysis; IQR, interquartile range; MRSA, methicillin-resistant *Staphylococcus aureus*; SD, standard deviation.

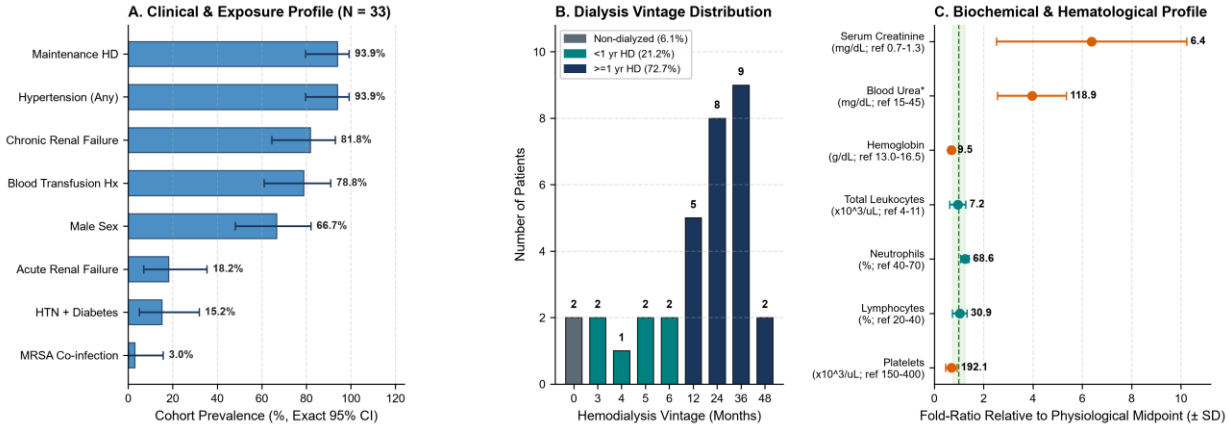

Figure 1. Clinical, dialysis vintage, biochemical, and hematological architecture of the study cohort (*N* = 33). (A) Cohort prevalence of renal failure subtypes, transfusion exposure, sex, and comorbidities with exact two-sided 95% Clopper–Pearson confidence error bars. **(B)** Discrete frequency distribution of

cumulative hemodialysis duration (0 to 48 months), illustrating predominance of long-term maintenance dialysis (>=12 months in 72.7% of patients). **(C)** Standardized biochemical and hematological profile plotted as mean ± SD fold-ratio relative to the physiological adult reference midpoint (shaded green band = normal physiological interval); numeric labels denote cohort raw means. *Blood urea mean reflects decimal-reconciled cohort value (118.9 mg/dL; range 25.60–199.00 mg/dL).

Renal Biochemical Severity and Hematological Cellular Competence

Biochemical profiling confirmed pronounced uremic retention across the cohort (**Table 2**, **Figure 1C**). Serum creatinine averaged 6.38 ± 3.86 mg/dL (range 0.60–12.70 mg/dL), representing an approximately 6.4-fold elevation above physiological adult reference midpoints. Blood urea concentrations ranged from 25.60 to 199.00 mg/dL with a standard deviation of 41.80 mg/dL (consistent with a cohort mean of ~118.9 mg/dL, recorded with a shifted decimal point as `1.189` in archival SPSS output; **Table 2** footnote). Consistent with impaired renal erythropoietin synthesis and chronic uremic inflammation, evaluable patients (n = 31) exhibited moderate normocytic-spectrum anemia, with a mean hemoglobin concentration of 9.49 ± 1.45 g/dL (range 6.50–13.70 g/dL), directly accounting for the high clinical reliance on allogeneic packed red blood cell transfusions.

In contrast to classically neutropenic or profoundly lymphopenic transplant cohorts, peripheral leukocyte indices demonstrated largely preserved quantitative cellularity (**Table 2**, **Figure 1C**). Mean total leukocyte count was 7.16 ± 2.44 × 10^3/µL (range 3.40–14.30 × 10^3/µL), with no patient exhibiting severe leukopenia (<3.0 × 10^3/µL). Differential leukocyte counts showed a mean neutrophil proportion of 68.64 ± 9.33% (range 54.00%–94.00%) and a mean lymphocyte proportion of 30.88 ± 8.92% (range 6.00%–45.00%). While the cohort mean neutrophil-to-lymphocyte ratio (~2.22) fell within physiological bounds, the wide individual span—extending down to 6.0% lymphocytes and up to 94.0% neutrophils (stress neutrophil-to-lymphocyte ratio >15)—reflected acute inflammatory stress in a subset of critically ill decompensated patients. Platelet counts averaged 192.12 ± 63.24 × 10^3/µL (range 62.00–328.00 × 10^3/µL), indicating mild uremic or sepsis-associated thrombocytopenia in isolated cases without severe hemorrhagic thrombocytopenic marrow failure.

Table 2. Renal biochemical and hematological parameters of enrolled patients with end-organ renal failure.

Laboratory Parameter	Evaluable n	Mean ± SD	Observed Min – Max	Adult Reference Interval	Clinical Interpretation
Serum Creatinine (mg/dL)	33	6.38 ± 3.86	0.60 – 12.70	0.70 – 1.30	Severe azotemia / ESRD retention
Blood Urea (mg/dL)[a]	33	118.9 ± 41.80[a]	25.60 – 199.00	15.0 – 45.0	Marked uremia across cohort
Hemoglobin (g/dL)[b]	31	9.49 ± 1.45	6.50 – 13.70	12.5 – 16.5	Moderate renal anemia (drives RBC Tx)
Total Leukocyte Count (×10^3/µL)	33	7.16 ± 2.44	3.40 – 14.30	4.00 – 11.00	Preserved systemic leukocyte count

Laboratory Parameter	Evaluable n	Mean ± SD	Observed Min – Max	Adult Reference Interval	Clinical Interpretation
Neutrophils (%)	33	68.64 ± 9.33	54.00 – 94.00	40.0 – 70.0	Normal mean; stress neutrophilia in subset
Lymphocytes (%)	33	30.88 ± 8.92	6.00 – 45.00	20.0 – 40.0	Normal mean; acute lymphopenia in subset
Platelet Count (×10³/µL)[c]	33	192.12 ± 63.24	62.00 – 328.00	150.0 – 400.0	Preserved mean; mild uremic TCP in subset

[a] Archival SPSS output recorded Min = 25.60 mg/dL, Max = 199.00 mg/dL, SD = 41.801 mg/dL, and printed mean string `1.189`, representing a 10^2 decimal-point transcription shift of 118.9 mg/dL. [b] Two specimens lacked automated hemoglobin readout (n = 31; tabular mean 9.488 ± 1.453 g/dL, rounded text summary 9.40 ± 1.42 g/dL). [c] Tabular mean `19212` reflects `192.12 × 10³/µL` (SD 63.24 × 10³/µL; range 62.00–328.00 × 10³/µL). *Abbreviations:* ESRD, end-stage renal disease; RBC Tx, red blood cell transfusion; TCP, thrombocytopenia.

Molecular Internal Control Validation and Qualitative HCMV PCR Surveillance

Genomic DNA extraction from 300 µL EDTA whole blood succeeded across all 33 clinical specimens. On 2% agarose gel electrophoresis, 33/33 patient DNA extracts (100.0%; exact 95% CI: 89.42%–100.00%) produced sharp 110 bp human *ACTB* (beta-actin) bands concordant with the human genomic positive control, while no-template negative controls remained completely blank. This confirmed unimpaired DNA template recovery and excluded false-negative PCR failure secondary to residual heme porphyrins, uremic solutes, EDTA chelation of Mg^{2+} ions, or thermal cycler malfunction.

On subsequent qualitative HCMV PCR testing (CinnaGen PR7836C), the kit positive control generated the expected 222 bp viral amplicon and negative reagent controls showed zero background amplification in all runs. However, **0 out of 33 patient whole-blood DNA samples (0.00%)** yielded a detectable 222 bp HCMV band under UV transillumination.

Exact Zero-Numerator Confidence Bounds, Subgroup Stratification, and Statistical Power

To quantify precisely what prevalence rates of whole-blood HCMV DNAemia are compatible with—or statistically excluded by—an observed proportion of `0/33`, exact small-sample confidence bounds were evaluated across all clinical strata (**Table 3**, **Figure 2A**). For the overall cohort (N = 33), the exact two-sided 95% Clopper–Pearson CI was **0.00%–10.58%**, the Wilson score 95% upper bound was **10.43%**, Hanley's rule-of-three upper bound was **9.09%**, and the exact one-sided 95% upper risk bound was **8.68%**. Within key high-exposure clinical subgroups, two-sided 95% Clopper–Pearson upper bounds were **11.22%** among active hemodialysis recipients (`0/31`), **12.77%** among chronic renal failure patients (`0/27`), **13.23%** among blood-transfused patients (`0/26`), and **14.25%** among patients on maintenance hemodialysis for >=12 months (`0/24`), whereas smaller strata such as acute renal failure (`0/6`; upper bound 45.93%) carried expectedly wide uncertainty.

Post-hoc power analysis (**Figure 2B**) demonstrated that a sample size of $N = 33$ provides **80% power** to detect at least one HCMV-positive patient if true underlying assay-detectable DNAemia prevalence is **>=4.76%**, **90% power** if prevalence is **>=6.74%**, **95% power** if prevalence is **>=8.68%**, and **99% power** if prevalence is **>=13.03%**. Consequently, exact two-sided binomial hypothesis tests (**Figure 2B**) decisively rejected equality between our renal failure cohort (`0/33`) and established HCMV reactivation rates in non-immunosuppressed ICU populations—including Jaber et al.'s medico-surgical ICU antigenemia rate of 16.88% (`40/237`; $P = 0.0040$) [12], Osawa and Singh's systematic review benchmark of 17.0% ($P = 0.0040$) [13], Li et al.'s pooled ICU meta-analytic prevalence of 27.0% ($P = 4.24 \times 10^{-5}$) [16], and Limaye et al.'s serial qPCR ICU incidence of 32.5% (`39/120`; $P = 4.93 \times 10^{-6}$) [14]. Conversely, `0/33` was completely consistent with international non-transplant hemodialysis PCR studies, including Pliquett et al. (`0/52` [0.0%]; $P = 1.00$) [26] and Kelishadi et al. (`4/149` [2.68%]; two-sided $P = 1.00$, one-sided $P = 0.407$) [28].

Table 3. Exact zero-numerator confidence bounds for whole-blood HCMV DNAemia across clinical risk strata (*ACTB*-validated extracts).

Clinical Risk Stratum	HCMV+ / Evaluable n	Point Prev. (%)	Clopper–Pearson 2-Sided 95% Upper (%)	Wilson Score 95% Upper (%)	Exact 1-Sided 95% Upper (%)	Rule-of-Three Upper (%)
Full End-Organ Renal Cohort	0 / 33	0.00	10.58	10.43	8.68	9.09
Maintenance Hemodialysis Subgroup	0 / 31	0.00	11.22	11.03	9.21	9.68
Hypertensive Comorbidity Subgroup	0 / 31	0.00	11.22	11.03	9.21	9.68
Chronic Renal Failure (CKD Stage 5)	0 / 27	0.00	12.77	12.46	10.50	11.11
Allogeneic Blood-Transfused Subgroup	0 / 26	0.00	13.23	12.87	10.88	11.54
Dialysis Vintage >= 12 Months	0 / 24	0.00	14.25	13.80	11.73	12.50
Male Patients	0 / 22	0.00	15.44	14.87	12.73	13.64
Dialysis Vintage >= 24 Months	0 / 19	0.00	17.65	16.82	14.59	15.79
Female Patients	0 / 11	0.00	28.49	25.88	23.84	27.27
Acute Renal Failure Subgroup	0 / 6	0.00	45.93	39.03	39.30	50.00

Note: Lower bound is 0.00% across all zero-event strata. Two-sided Clopper–Pearson upper bound = `1 - 0.025^(1/n)`; exact one-sided 95% upper bound = `1 - 0.05^(1/n)`; Hanley–Lippman-Hand rule-of-three = `3/n` [31,32].

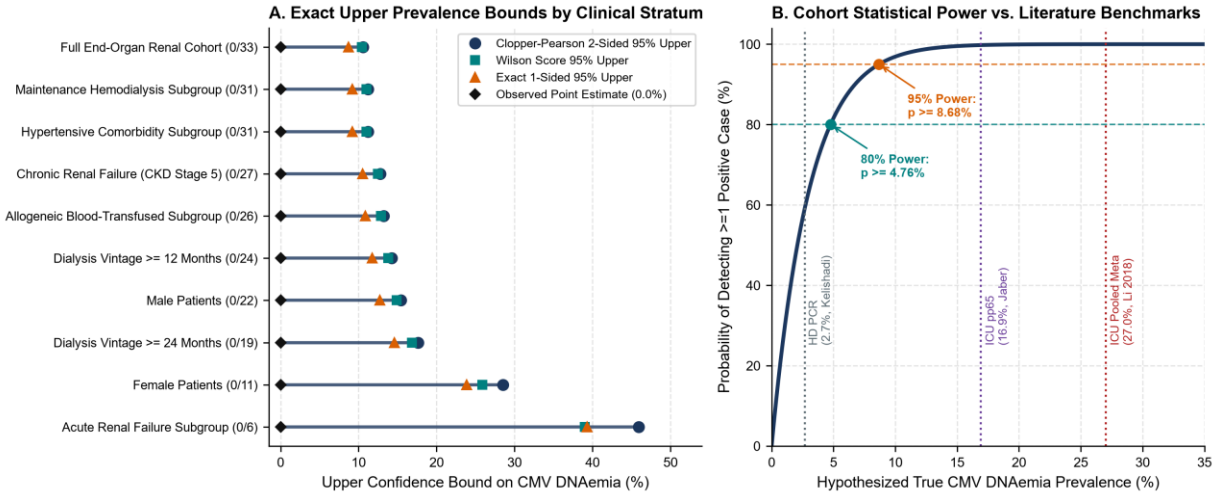

Figure 2. Frequentist exact zero-numerator bounds and cohort statistical detection power. (A) Comparison of two-sided 95% Clopper–Pearson, Wilson score, and exact one-sided 95% upper confidence limits on HCMV DNAemia across clinical and exposure strata. **(B)** Exact binomial detection power curve (`1 - (1 - p) ^33`) illustrating >=95% power to detect true high-level viremia >=8.68% and >99.9% power against typical ICU qPCR reactivation benchmarks (27.0%–32.5%), contrasted against low-prevalence maintenance hemodialysis PCR studies (2.7%).

Bayesian Posterior Updating and Stoichiometric Analytical Sensitivity Modeling

Bayesian Beta-Binomial conjugate analysis (**Table 4**, **Figure 3A**) further refined estimates of underlying high-level HCMV DNAemia. Under an uninformative Jeffreys prior `Beta (0.5, 0.5)`, observing `0/33` yielded a posterior `Beta(0.5, 33.5)` distribution with a posterior median of **0.68%** (mean 1.47%; 95% credible interval [CrI]: **0.001%–7.28%**) and a **93.52% posterior probability** that true assay-detectable prevalence is <5.0%. Incorporating an informative prior synthesized from prior hemodialysis PCR cohorts (`Beta(4.5, 197.5)`) [26,28] yielded a tightly constrained posterior median of **1.78%** (mean 1.91%; 95% CrI: **0.58%–4.01%**), corresponding to a **99.54% probability** that true systemic viremia in maintenance dialysis patients lies below 5.0%. Even under a skeptical ICU-derived prior centered at 27.0% (`Beta (2.7, 7.3)`), our `0/33` data induced marked downward shrinkage to a posterior median of **5.61%** (95% CrI: **1.19%–15.12%**).

Finally, stoichiometric reaction modeling clarified the analytical boundary separating qualitative end-point gel PCR from modern WHO-standardized real-time qPCR (**Figure 3B**). Because 300 µL of EDTA whole blood was eluted into 50 µL and 5.0 µL of eluate was added per 25 µL PCR tube, each qualitative amplification reaction interrogated exactly **30 µL (0.030 mL)** of original whole-blood equivalent. Given that single-round 35-cycle agarose/ethidium bromide visualization reproducibly requires ~15–25 intact viral target copies per reaction tube to exceed the visual fluorescence threshold (`LOD95`), 15–25 copies divided by 0.030 mL corresponds to an analytical 95% detection threshold of **~500–833 copies/mL** (probit midpoint `C50` ~250–300 copies/mL). While this sensitivity readily captures clinically significant high-grade HCMV disease (`>1,000–2,500 copies/mL`) [14,30], low-grade or transient viral blips (`20–400 IU/mL`)—which account for over half of early reactivations detected by serial real-time qPCR (`LOD95` ~20–50 IU/mL) [14,29]—fall within a diagnostic blind zone (**Figure 3B**, shaded band).

Table 4. Bayesian Beta-Binomial conjugate posterior estimates of underlying HCMV DNAemia prevalence given `0/33` observed events.

Prior Specification	Prior Beta(α_0, β_0)	Posterior Beta(α, β)	Posterior Mean (%)	Posterior Median (%)	95% Equal-Tailed CrI (%)	Pr(True Prev < 5%)
Jeffreys Uninformative	Beta(0.5, 0.5)	Beta(0.5, 33.5)	1.47	0.68	0.001 – 7.28	93.52%
Uniform Flat	Beta(1.0, 1.0)	Beta(1.0, 34.0)	2.86	2.02	0.074 – 10.28	82.52%
Hemodialysis PCR Prior[a]	Beta(4.5, 197.5)	Beta(4.5, 230.5)	1.91	1.78	0.580 – 4.01	99.54%
ICU Meta-Analytic Prior[b]	Beta(2.7, 7.3)	Beta(2.7, 40.3)	6.28	5.61	1.193 – 15.12	42.82%

[a] Empirical prior constructed from international maintenance hemodialysis PCR cohorts: Pliquett et al. (`0/52`) [26] and Kelishadi et al. (`4/149`) [28] with 0.5 continuity prior. [b] Weakly informative prior (`n_eff = 10`) centered at the 27.0% pooled ICU reactivation rate reported by Li et al. [16]. *Abbreviation:* CrI, Bayesian credible interval.

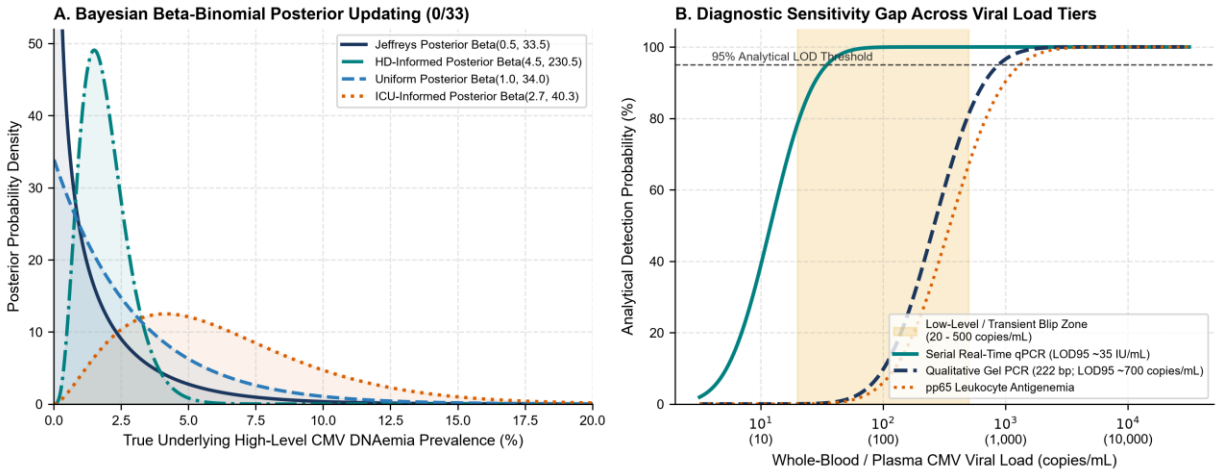

Figure 3. Bayesian posterior density updating and stoichiometric diagnostic sensitivity gap
Posterior probability density functions for underlying high-level whole-blood HCMV DNAemia after updating four prior distributions with `0/33` observed cases. **(B)** Probit analytical detection curves comparing WHO-calibrated real-time qPCR (`LOD95` ~35 IU/mL), single-round qualitative agarose-gel PCR (222 bp amplicon from 30 µL blood equivalent; `LOD95` ~700 copies/mL), and pp65 leukocyte antigenemia across $\log_{10}$ viral load tiers. Shaded yellow zone highlights low-level transient DNAemia (20–500 copies/mL) undetected by conventional qualitative end-point PCR.

Discussion

This molecular surveillance study demonstrates that among hospitalized non-transplant Pakistani adults with severe acute or chronic end-organ renal failure—93.9% of whom were maintained on intermittent hemodialysis (median vintage 24 months) and 78.8% of whom had received allogeneic blood

transfusions—none (`0/33`; exact 95% CI: `0.00%–10.58%`) exhibited detectable whole-blood HCMV DNAemia on *ACTB*-validated qualitative PCR. Rather than representing an anomalous negative result, our findings—when interpreted through exact binomial probability theory, Bayesian synthesis, and reaction stoichiometry—resolve an important epidemiological distinction between uremic renal dysfunction and acute septic immunoparalysis while establishing practical diagnostic thresholds for nephrology and critical care laboratories in hyperendemic LMIC settings.

First, our observation of 0% whole-blood HCMV DNAemia aligns closely with rigorous molecular investigations of maintenance hemodialysis populations internationally and contrasts sharply with serological studies relying solely on anti-HCMV IgM [25–28]. In a landmark German cohort of 52 maintenance hemodialysis patients, Pliquett et al. [26] noted anti-HCMV IgM reactivity in 15.4% (`8/52`) of patients yet detected **0/52 (0.0%)** cases of systemic HCMV DNAemia using highly sensitive real-time PCR (detection limit 20 copies/mL), demonstrating that polyclonal B-cell activation, rheumatoid-factor cross-reactivity, and uremic retention frequently generate false-positive IgM signals in dialysis patients. Similarly, Kelishadi et al. [28] detected plasma HCMV DNA by conventional PCR in only **2.68% (`4/149`)** of Iranian hemodialysis patients despite high regional seroprevalence. Synthesizing our data (`0/33`) with these molecular cohorts via Bayesian updating indicates a 99.5% probability that true systemic HCMV DNAemia in non-transplant hemodialysis patients lies below 5% (posterior median 1.78%).

Second, our statistical benchmark analysis (`P = 0.004` vs. 16.9% pp65 antigenemia [12]; `P < 0.0001` vs. 27.0%–32.5% ICU qPCR reactivation [14,16]) underscores a fundamental immunobiological difference between **chronic uremic immunosenescence** and **acute critical-illness immunoparalysis** [18–22]. Although end-stage renal disease impairs naive T-cell priming and vaccine responsiveness [21,22], chronic HCMV infection itself drives massive oligoclonal inflation of effector-memory HCMV-specific CD8+ and CD4+ T lymphocytes in seropositive uremic adults [22]. As evidenced by our hematological data (**Table 2**), non-transplant renal failure patients maintained preserved circulating leukocyte (`7.16 ± 2.44 × $10^3$/μL`) and lymphocyte (`30.88 ± 8.92%`) counts, sustaining sufficient cytotoxic surveillance to prevent exponential systemic lytic replication. By contrast, fulminant HCMV reactivation in general ICUs typically requires a 'two-hit' cascade: intense systemic pro-inflammatory transcriptional activation of the viral MIEP (triggered by septic shock, major trauma, or extensive thermal burns) followed by profound lymphocyte apoptosis, HLA-DR downregulation, and prolonged (>10–15 days) mechanical ventilation [4,14,18–20].

Third, the absence of detectable HCMV viremia despite a **78.8% blood transfusion rate** highlights the paradoxical epidemiology of transfusion-transmitted CMV (TT-CMV) in hyperendemic regions [5,7,8,23,24]. In low-seroprevalence Western cohorts, transfusion of non-leukoreduced cellular blood products from seropositive donors into CMV-seronegative immunocompromised recipients carries a substantial risk of primary viremic disease [23,24]. In Pakistan, however, where 90.2%–97.8% of adult blood donors and recipients already harbor latent HCMV and high-avidity neutralizing anti-glycoprotein B/pentameric-complex IgG antibodies [7,8], pre-existing recipient humoral and memory T-cell immunity rapidly clears small inocula of latently infected donor leukocytes before high-copy systemic DNAemia can be established [6,24]. Nevertheless, because hemodialysis patients are prospective kidney transplant candidates in whom donor-derived strain superinfection or HLA-alloimmunization carries deleterious post-transplant consequences, implementation of pre-storage universal leukoreduction (`<1–5 × $10^6$` residual leukocytes/unit) remains an essential transfusion-medicine priority in LMIC renal centers [7,23,24].

Fourth, our stoichiometric LOD analysis (**Figure 3B**) provides a critical methodological lesson for clinical virology laboratories in developing countries. Conventional single-round qualitative agarose-gel PCR interrogating 30 μL of whole-blood equivalent exhibits a practical `LOD95` of ~500–830 copies/mL, roughly 15- to 25-fold less sensitive than automated real-time qPCR calibrated to the 1st WHO International Standard for HCMV (`LOD95` ~20–50 IU/mL) [29,30]. Moreover, single-timepoint cross-sectional sampling early during inpatient stabilization misses late-onset reactivation, which peaks at a

median of 12–18 days after ICU admission [12,14,15]. Importantly, however, landmark clinical studies indicate that low-level transient DNAemia (`<500–1,000 copies/mL`) rarely represents invasive end-organ viral cytopathology and does not benefit from empiric ganciclovir therapy—which carries severe dose-limiting myelotoxicity and nephrotoxicity in patients with renal insufficiency [14,17,30]. Thus, while our qualitative assay securely excluded high-burden HCMV viremia (`>=1,000 copies/mL`) up to an exact one-sided 95% upper bound of 8.68%, clinicians seeking to diagnose true late-onset HCMV disease in refractory ICU sepsis or post-transplant settings should utilize serial WHO-standardized quantitative qPCR paired with viral load kinetic thresholds [10,29,30].

Strengths and Limitations

Key strengths of this study include: (i) rigorous endogenous *ACTB* amplification verification in 100% of clinical EDTA whole-blood extracts, eliminating false-negative inhibition artifacts common in uremic/anemic blood matrices; (ii) comprehensive concurrent profiling of uremic biochemistry, leukocyte differentials, transfusion history, and granular dialysis vintage; and (iii) transparent application of exact Clopper–Pearson, Wilson, Hanley–Lippman-Hand, Bayesian Beta-Binomial, and stoichiometric LOD frameworks to avoid the statistical pitfalls of zero-numerator misinterpretation [31,32]. Several limitations warrant consideration. First, the sample size (N = 33) and consecutive non-random single-region recruitment limit precision for rare viremic events (<3%–5%), as explicitly quantified by our 10.58% two-sided upper confidence bound. Second, baseline anti-HCMV IgG/IgM serostatus and donor blood unit serostatus were not concurrently assayed, although contemporary Lahore donor data confirm ~97.8% adult IgG seropositivity [7]. Third, blood sampling was cross-sectional rather than longitudinal across weeks 2–4 of ICU stay, and respiratory tract specimens (bronchoalveolar lavage or tracheal aspirates) were not tested to assess compartmentalized pulmonary reactivation. Finally, minor clerical recording anomalies in archival laboratory logs (two missing hemoglobin entries and a decimal-point shift in tabular mean urea) were transparently audited and reported without data imputation.

Conclusions

Active high-level whole-blood HCMV DNAemia was absent (`0/33`; exact two-sided 95% CI: `0.00%–10.58%`; Bayesian hemodialysis-informed posterior median: `1.78%`) among hospitalized non-transplant Pakistani adults with acute and chronic end-organ renal failure, despite prolonged maintenance hemodialysis and a 78.8% prevalence of non-leukoreduced blood transfusion. Preserved circulating lymphocyte counts and near-universal background HCMV immunity likely restrain systemic lytic viral replication in uremic patients lacking profound iatrogenic immunosuppression or prolonged septic shock. Routine untargeted screening of non-transplant renal failure inpatients using single-round qualitative gel PCR is not cost-effective; instead, LMIC diagnostic laboratories should deploy serial WHO-calibrated quantitative real-time PCR selectively for critically ill patients with prolonged (>14 days) mechanical ventilation, refractory septic organ failure, or pre-/post-renal transplant surveillance, coupled with pre-storage leukoreduction of transfused blood components.

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