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RECEIVED 03 September 2025

ACCEPTED 20 October 2025

PUBLISHED 31 October 2025

CITATION

Marascio N, Pavia G, Mazzei C, Pantanella M,
Giorgio E, Manno M, Gigliotti S, Barreca GS,
Peronace C, Matera G and Quirino A (2025)
Human *Cytomegalovirus* infection in the era
of vaccine development: case series of
immunocompromised patients.
Front. Virol. 5:1698340.
doi: 10.3389/fviro.2025.1698340

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Human *Cytomegalovirus* infection in the era of vaccine development: case series of immunocompromised patients

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Introduction: Human Cytomegalovirus (CMV) infection represents a significant health burden, particularly for immunocompromised patients, including solid-organ transplant (SOT) recipients and people living with human immunodeficiency virus (PLWH). Despite the availability of antiviral prophylaxis and treatment, prolonged therapy can lead to viral drug resistance, complicating disease management. In this study, we present a series of CMV cases in immunocompromised patients, including two SOT recipients and one PLWH patient, focusing on microbiological data, clinical presentation, and therapeutic management.

Methods: CMV serostatus and DNA viral load were carried out by Chemiluminescence Immunoassay (CLIA) and quantitative real-time PCR to monitor patient follow-up.

Results: The three patients had CMV reactivation following an immunocompromised status. The prompted antiviral treatments determined the viral infection resolution, despite CMV-related complications worsening clinical outcomes.

Discussion: The development of a safe and effective CMV vaccine represents a needed challenge, especially for individuals at high risk of severe CMV-related complications. However, it is difficult to achieve high CMV variability. Our findings contribute to the ongoing discussion on the importance of developing vaccines to mitigate CMV-related morbidity in vulnerable populations.

KEYWORDS

human cytomegalovirus (CMV), case series, vaccine, genetic variability, antiviral therapy

1 Introduction

The Human Cytomegalovirus (CMV), *Herpesviridae* family, *Betaherpesvirinae* subfamily, is a widespread *Herpesvirus* infecting a significant portion of the global population (1). It is known for the ability to establish lifelong latent infections after primary exposure, which predominantly occur during childhood or early adulthood (1). CMV exhibits considerable genetic diversity compared to other human *Herpesviruses*, largely due to recombination and coinfection events (2–4). The viral seroprevalence ranges from 45% to 100% worldwide, with specific IgG antibody positivity in up to 60% of adults in industrialized countries and over 90% in individuals with lower socioeconomic conditions (5). Typically, primary infection is asymptomatic or causes mild, mononucleosis-like symptoms in immunocompetent individuals (1).

However, CMV poses severe health risks to immunocompromised patients (6) and women who contract primary CMV infection during the first trimester of pregnancy (7, 8). Currently, congenital CMV infection remains the leading cause of non-genetic sensorineural hearing loss and a significant contributor to neurodevelopmental disorders (9). In solid-organ transplant (SOT) recipients, hematopoietic stem cell transplantation (SCT) recipients, and people living with human immunodeficiency virus (PLWH) (9), the virus may reactivate, leading to illnesses that include pneumonia, gastroenteritis, and retinitis (6). Additionally, reinfection with a new CMV strain can occur through direct contact with an infectious individual (1). Although the target populations and the mechanisms of vaccine delivery are not yet defined, it is well known that children born with congenital infection and immunocompromised subjects are the two groups of patients suffering the most serious consequences of CMV contact. Thus, the most suitable vaccination targets could be transplant recipients and pregnant women or seronegative women of childbearing age to prevent congenital infection (10, 11). Antiviral prophylaxis or treatments that prevent serious outcomes of diseases are currently available for SOT, HSCT, and PLWH patients. However, prolonged antiviral therapies can accumulate mutations in CMV DNA hotspots, conferring resistance to antivirals and consequently causing therapy failure (12). The different clinical scenarios (primary infection, reactivation, and reinfection) and treatments cause virus variability (12–14). In particular, the genes encoding envelope glycoproteins may segregate by genetic assortment into distinct genotypes, which have been reported to influence viral virulence, immune response, and disease outcome (15–17). Considering the key role of envelope glycoproteins in CMV infection and antibody (Ab)-mediated neutralization immunity, the development of an effective and safe vaccine for CMV that can provide broad protection against multiple genotypes, without causing severe complications, is considered a major challenge that would, however, address a relevant and yet unsatisfied medical need (18, 19). In this study, we report the clinical and therapeutic management of CMV infection in immunocompromised patients, specifically two SOT recipients and one PLWH. By reviewing antiviral treatments, dosing strategies, and patient outcomes, we reported the effectiveness of current CMV management approaches. Additionally, to address the discussion on the importance of vaccination in immunocompromised populations, we started with

the main virological characteristics and infection control, going through the current vaccine research.

2 Materials and methods

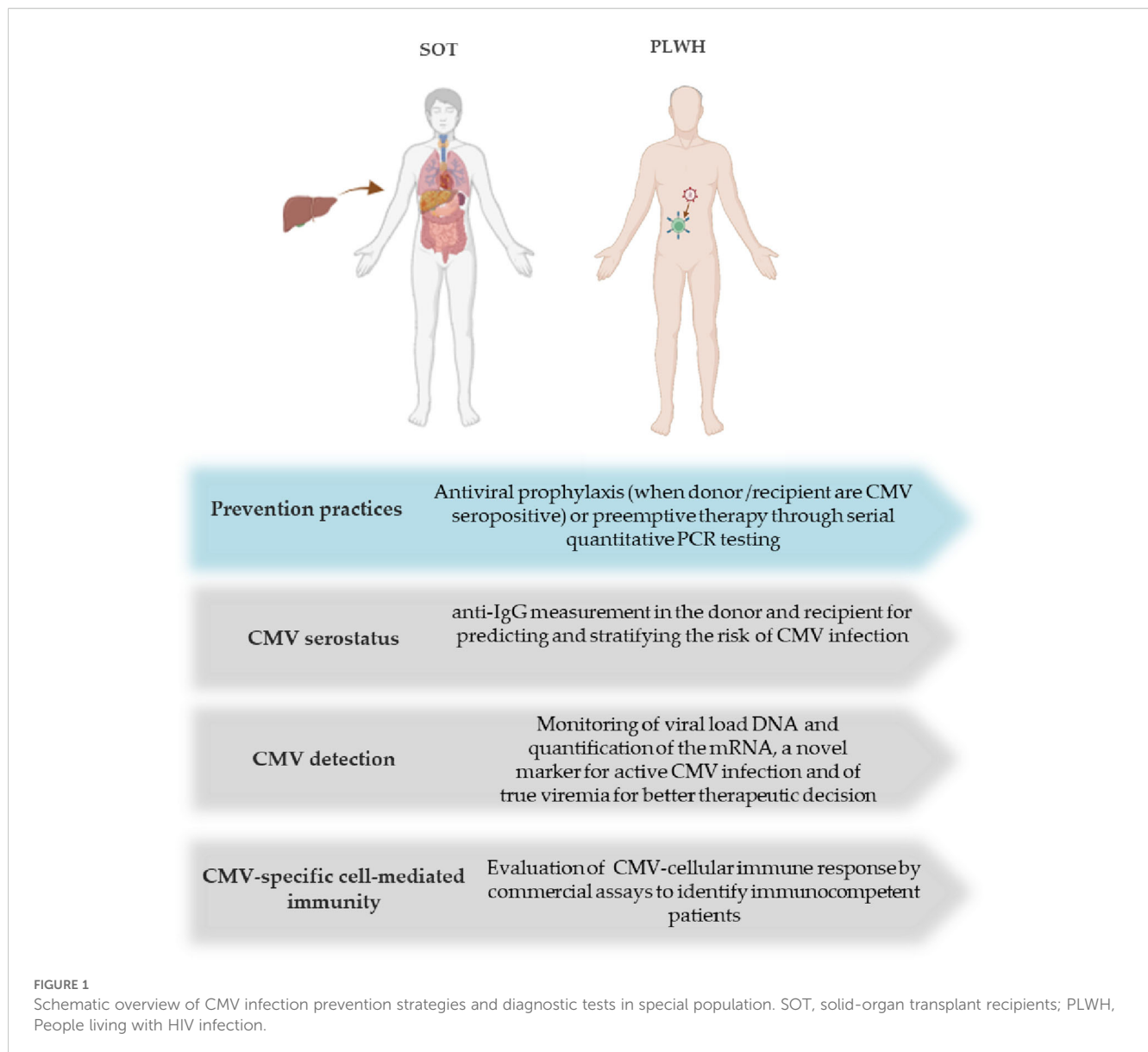
2.1 Study design

Accurate diagnosis of CMV and practices preventing CMV infection are of paramount importance to several special populations, such as SOT recipients and PLWH. The prevention strategies and diagnostic tests performed in special populations infected with CMV are summarized in Figure 1.

CMV serostatus, viral genomic detection (DNA or mRNA), and specific cell-mediated immunity (CMV-CMI) are important factors that determine outcomes after SOT and in PLWH under antiretroviral therapy (ART) (20, 21). To improve the post-operative outcome of transplantation, it is crucial to shift the focus of CMV detection to the donor and achieve early diagnosis, as well as implement effective preventive and therapeutic measures. For CMV prevention, there are two main methods, the first one is universal prophylaxis, which involves giving antiviral medication at prophylaxis dose when either the donor and/or the recipient is seropositive for CMV. The second one is preemptive therapy, which is defined as serial testing done weekly, through quantitative PCR, for the first few months after transplant or after treatment of rejection with a treatment dose of antiviral therapy (22).

2.2 Antiviral treatment and candidate vaccine

At present, the United States Food and Drug Administration (FDA) has approved six drugs, which target specific genomic regions, to treat or prevent CMV disease (11). Two main different approaches, based on patient CMV immune status, clinical conditions, risk factors, and co-morbidities, were adopted for the prevention of primary, reactivated, or recurrent CMV infection: universal prophylaxis and/or pre-emptive therapy. Universal prophylaxis makes it possible to maintain viral latency for CMV infection or reactivation in high-risk SOT and allo-HSCT recipients, as well as in PLWH (23). However, this therapeutic strategy is administered to all patients, even those in whom CMV cannot reactivate, thus needlessly exposing them to side effects. Moreover, this approach does not prevent the virus from reactivating after the discontinuation of the prophylactic therapy (24). Pre-emptive therapy is applied to asymptomatic CMV-infected patients with positive viremia diagnosed by molecular screening tests. Based on the CMV viral load measured for 3 or 6 months, antiviral agents are to be administered weekly (25). To avert the risk of CMV reactivation after prophylaxis discontinuation, a hybrid approach (prophylactic approach for 3 to 6 months, followed by pre-emptive therapy) has also been evaluated (26, 27). In 2017, the introduction of letermovir (LTM), a new anti-CMV molecule that binds to the components of the viral



terminase complex (UL51, UL56, and UL89) to block CMV DNA processing and viral particle packaging, provided a novel prophylactic approach (28, 29). In a randomized controlled trial, LTM prophylaxis proved to be superior to pre-emptive therapy in reducing the clinical picture of CMV infections and enhancing 24-week survival rates (30). However, prolonged and repeated use of anti-CMV therapies (which typically last months) can lead to the accumulation of mutations in target regions of the CMV genome; this, in turn, confers resistance to antivirals (Table 1) (29–40).

The CMV management guidelines for patients with persistent symptoms of disease or rising/relapsing viremia recommend genotypic resistance testing (11). Several candidate vaccines for CMV that aim to prevent congenital and post-transplant infections in immunocompromised people, as well as in healthy subjects, are currently in development (Figure 2).

The development of CMV vaccines began in the 1970s when two strains of the virus, Towne and AD169, were attenuated and

used as active immunoprophylaxis in solid organ transplant recipients (41). These strains have several modifications in an area of the genome that consists of sequences spanning CMV ORFs UL128–151. Specifically, UL128, UL130, and UL131 direct the synthesis of three polypeptides that are constituents of the pentameric complex (PC) required for efficient viral tropism in most epithelial and endothelial cell types (10). Despite initial results being seemingly promising, statistical analyses revealed that protection against infection was not significant (42). V160 is the first attenuated vaccine designed to express the PC to be constructed on the backbone of the AD169 strain. V160 can propagate in epithelial cell lines only in the presence of Shield-1, a synthetic stabilizing ligand. In the absence of this ligand, the fusion protein is rapidly degraded and viral replication is inhibited (43). Therefore, given that the Shield-1 ligand does not exist in nature, the V160 attenuated virus should be unable to revert to a replication-competent virus, ensuring an excellent safety profile for this

TABLE 1 Available drugs and related resistance mutations within genomic target regions.

Drug	Genomic target and action	Resistance mutation	References
ganciclovir (GCV) valganciclovir (VGCV)	Blocking of viral DNA replication.	Mutations in UL97 gene (codons 363 to 698) and/or in the UL54 gene (codons 184 to 1017).	(31, 32)
foscarnet (FOS)	Binding of UL54 site viral polymerase, halting DNA chain elongation.	Mutations in UL54 gene (codons 555– 600), palm and finger (codons 696–981).	(33–35)
cidofovir (CDV)	Inhibition of incorporation of deoxycytidine triphosphate into viral DNA by UL54 viral polymerase.	Mutations in UL54 (codons 301 – 987) and deletion at codons 981– 982.	(36–38)
letermovir (LMV)	Inhibition of cleavage of long DNA concatamers, resulting in the production of non-infectious viral particles.	Mutation in UL51 (amino acid changes P91S) and UL89 (amino acid changes N320H, D344E and M359I) enzyme complex encoding-genes.	(28, 29)
maribavir (MBV)	Inhibition of the UL97 kinase enzyme, blocking the assembly of the infectious viral progeny.	Mutation in UL97 gene (amino acid changes T409M, H411Y, and C480F)	(39, 40)

vaccine. In a Phase I study conducted between 2013 and 2017, it was demonstrated that the V160 vaccine can induce neutralizing antibody and T cell responses. Furthermore, after a new dose, there is an increase in cell-mediated immune response to CMV (44). The glycoprotein gB is one of several glycoproteins expressed in the viral envelope. This protein works in combination with both the gH/gL complex to facilitate viral entry into human fibroblasts and with the PC to enter epithelial and endothelial cells (45). A phase I randomized trial was conducted involving a CMV vaccine based on recombinant gB with an adjuvant, MF59, an oil-in-water emulsion of squalene. This study demonstrates that immunization of healthy adults achieved with a subunit CMV vaccine combined with an adjuvant can induce an immune response to the gB neutralizing antibody (46). A Phase 2 trial (NCT00299260) measured, after the administration of the gB/MF59 vaccine, antibody titers and CMV viremia in kidney or liver transplant patients. The results showed a significant increase in the gB-binding antibody titer one month after the second vaccine dose. Furthermore, an increase in neutralizing antibody titers was measured after a similar amount of time in seropositive vaccine recipients. Similarly, seronegative organ recipients who had received the vaccine and had had seropositive organ donors demonstrated reduced viremia. This viremia had a post-transplantation duration that was inversely correlated to gB antibody titers (47). An issue to consider in the gB recombinant subunit vaccine is that the ability to induce immunity to one strain of CMV clearly does not mean immunity to all strains of CMV (48). According to the FDA, DNA vaccines are purified plasmid preparations containing one or more DNA sequences capable of inducing and/or promoting an immune response against a pathogen (49). After preclinical, the ASP0113 contains two plasmids encoding pp65 and gB and is administered with two adjuvants. A phase I clinical trial evaluated the safety of ASP0113 in CMV seropositive and seronegative immunized individuals. Vaccination of seronegative subjects elicited pp65- and gB-specific T-cell responses in addition to gB antibody responses, while seropositive vaccinated groups showed increases only in pp65-specific T-cell responses (50). The second clinical trial of

ASP0113 included the vaccination of allogeneic HSCT adult recipients, who then exhibited a significant reduction in viral load endpoints and increased frequencies of pp65-specific interferon- γ -producing T cells (51). Two similar studies evaluating the safety and efficacy of this DNA vaccine in solid organ transplant recipients (NCT01974206) and dialysis patients (NCT02103426) were recently completed. Transplant recipients were randomized (1:1) to receive 5 doses of ASP0113 and showed no statistically significant difference between the ASP0113 group and the placebo group. ASP0113 demonstrated a safety profile similar to the placebo in the prevention of CMV viremia in this CMV-seronegative kidney transplant population. A trivalent, non-adjuvanted DNA vaccine trial (VCL-CT02) is currently in progress. These studies (NCT00370006 and NCT00373412) include the IE1 T-cell target in addition to the gB and pp65 coding sequences and were conducted in CMV seronegative subjects vaccinated intramuscularly or intradermally, followed by Towne immunization. Furthermore, Inovio has recently been developing SynCon, an alternative nucleic acid-based vaccine technology. This model is based on an extensive sequence analysis of the antigen of several target pathogens, in which the most conserved, or dominant, amino acid in the antigen gene sequence is identified. Then, a consensus gene sequence is synthetically created and inserted into a DNA plasmid to create the testing vaccine (52). In addition to DNA vaccines, several RNA vaccines are also being studied, such as an alternative nucleic acid-based vaccine technology. An RNA vaccine uses a synthetic copy of a natural pathogen messenger RNA (mRNA) and leads to the immune system producing responses against its corresponding antigen, using lipid nanoparticles for delivery; these particles can protect the RNA strands and facilitate their absorption into the cells. Several preclinical studies have been carried out with excellent results (53). A CMV vaccine that uses a recombinant vector works by being composed of one or more antigens, which are delivered via a viral vector that is capable of infecting human cells and of expressing the viral proteins without establishing a productive infection (10). One of the candidate vaccines tested in clinical trials was a vaccine called Triplex, based on a modified Vaccinia Ankara encoding three immunodominant

	Vaccine	Target population	Pre-clinical	Phase 1	Phase 2	Phase 3
 Live Attenuated Vaccine	Towne NCT00201448	Seronegative Women				
	V160 NCT03486834	Healthy Donor				
 Recombinant subunits	VBI 1501A NCT02826798	Healthy Donor				
	gB/MF59 NCT00125502	Healthy adolescent Female and post partum women				
 Recombinant Viral Vectors	MVA-Triplex NCT05099965 NCT02506933	Adults With CMV and PLWHA, seropositive HSCT recipients				
 DNA or RNA	mRNA1647 NCT05085366 NCT05683457	Healthy Donor, SOT and HSCT recipients				
	ASP0113 NCT01877655	Seropositive recipients undergoing HSCT				
 Plasmid based	VCL-CB01 NCT00285259	Donors and recipients undergoing HSCT				

FIGURE 2

Summary of vaccines in completed or underway clinical trials. Vaccine and Clinical Trial Registration number (NCT identifier) were related to target population and development phase. SOT, solid-organ transplant recipients; HSCT, allogeneic hematopoietic stem cell transplant.

CMV antigens, pp65, IE1-exon4, and IE2-exon5. Triplex was tested in a Phase I trial evaluating the CMV serological status at different points in time by using three progressively higher doses of the vaccine (54). Based on these results, a Phase II clinical trial was started in 2015, in which CMV-seropositive HSCT recipients received Triplex two days after HSCT (NCT02506933), and then in 2021, Triplex was evaluated in adults with CMV infection and PLWH (NCT05099965). The first trial confirmed that, in patients who received the Triplex vaccine, the risk for a significant CMV event during the first 100 days after transplant was reduced by half, while the second trial is still ongoing (55). A novel strategy for a vaccine candidate was the use of virus-like particles (VLP), protein structures that mimic viruses without a viral genome. One candidate VLP vaccine against CMV was based on the production of VLP in mammalian cells encoding the truncated sequence of the gB extracellular portion fused with the TM and cytoplasmic domains of the vesicular stomatitis virus (VSV) G protein. In pre-clinical studies, this formulation, named CMV gB-G, demonstrated evidence of trimeric expression of the gB-G ectodomain, which is capable of eliciting higher epithelial neutralizing antibody titers compared to the full-length monomeric gB antigen (56). The high intra-host and inter-strain genetic diversity, together with frequent mixed and coinfections,

can substantially reduce the generalizability and real-world effectiveness of CMV candidate vaccines. Human CMV infection sustained by a mixture of genetically distinct strains within the same host, as reported by haplotype reconstruction and longitudinal case series, and recombination events could undermine strain-specific immunity and vaccine-induced protection (57). Case series of primary and congenital infections reported the multiple gB detection of different genotypes, as well as reinfection with heterologous strains despite prior immunity, indicating vaccines protection gaps targeting a single antigen or specific strain (58). Coinfection with multiple CMV genotypes was documented in transplant recipients and correlated to delayed antiviral immune reconstitution and prolonged viremia (59). CMV vaccine trials must account for mixed viral strain infections, regional strain variability, and microorganism coinfection when correlates of protection and clinical endpoints are evaluated (60, 61).

2.3 Routinely diagnose

Chemiluminescent immunoassay (CLIA) was performed to detect CMV Immunoglobulin M (IgM) and/or Immunoglobulin G (IgG) antibodies by LIAISON[®] system (Diasorin S.p.A, Italy).

Quantitative viremia was evaluated by NUCLESENS EASYMAG (BIOMERIEUX, Italy) and CMV ELITE MGB KIT QUANTITATIVE (ELITTECH GROUP S.P.A, Turin, Italy).

3 Case series of immunocompromised patients

3.1 Clinical case 1: CMV reactivation in PLWH recipient

A 36-year-old male was admitted to the “Renato Dulbecco” University Hospital of Catanzaro from August 05 to October 10, 2024. Medical history included HIV infection, first diagnosed in 2012, recurrent prostatitis, anorexia-related malnutrition, and previous AntiRetroviral Treatment (ART) regimens with poor adherence. Currently, the patient is on Highly Active AntiRetroviral Therapy (HAART) treatment to bictegravir/emtricitabine/tenofovir (50 + 200 + 25 mg cp; 1cp/die), exhibiting poor compliance. In the first evaluation, the immune status was moderately compromised, with a CD4+ T-cell count of 458/ μ L (26.5%, R: 0.45). The patient presented with fever (37.5 °C), mandibular pain with hypoesthesia and paresthesia along the second and third branches of the trigeminal nerve, and a pruritic erythematous rash on the trunk and extremities. The clinical presentation as well as current/past seroimmunological investigations, were consistent with secondary syphilis (rapid plasma reagin titer of 1:512). Additionally, quantitative real-time-RT-PCR revealed HIV RNA relapse from <20 to 23,600 copies/mL, with genotypic drug resistance sequencing confirming sensitivity to all antiretroviral drug classes. Diagnostic imaging demonstrated osteoarthritis involving the left mandibular condyle and temporal bone, which needed surgical management for recurrent temporomandibular joint dislocation. Throughout the hospitalization, the patient exhibited severe malnutrition, sarcopenia, and persistent gastrointestinal symptoms, including mucorrhea and poorly formed stools. A colonoscopy demonstrated chronic nonspecific inflammation, benign lymphoid hyperplasia, and anal condyloma. Biopsies revealed no evidence of malignancy. On August 28, CMV reactivation was detected with an initial DNA viral load of 1,929 copies/mL. It was promptly initiated with intravenous ganciclovir at a dose of 500 mg every 24 hours. The treatment was continued for three weeks, resulting in a progressive reduction of CMV DNAemia, until 192 copies/mL on October 10, 2024 (Figure 3). The immunological condition was re-evaluated, showing a decrease of the CD4+ T-cell count of 407/ μ L (26.5%, R: 0.38). HIV viral suppression (HIV-RNA <20 copies/mL) was achieved by directly observed therapy (DOT) with bictegravir/emtricitabine/tenofovir on September 6, 2024. However, adherence to HAART therapy remained suboptimal, leading to another virological relapse (HIV RNA viral load 7,540 copies/mL) on October 14, 2024. The patient’s hospital course was complicated by episodes of candidemia and a bloodstream infection caused by *Klebsiella pneumoniae* with New Delhi metallo-beta-lactamase (NDM) resistance phenotype. Caspofungin at a dose of 50 mg daily was administered for 15 days. For the NDM *K. pneumoniae* infection,

cefiderocol (2g x 3/die) and fosfomycin (4g x 4/die) were administered until September 11, 2024. Further microbiological investigations excluded other opportunistic pathogens, including *Cryptococcus neoformans*. Due to severe anorexia and malnutrition, parenteral nutrition was initiated to stabilize the patient’s nutritional status (Table 2).

3.2 Clinical case 2: CMV reactivation in kidney transplant patient

A 25-year-old woman was admitted to the Nephrology-Dialysis Unit of the “Renato Dulbecco” University Hospital of Catanzaro from April 13 to 15, 2023, with symptoms of dyspnea and fever. The patient’s medical history included opsismodysplasia, chronic obstructive pulmonary disease, chronic pancreatitis, and retinitis pigmentosa. In October 2017, the patient was diagnosed with initial renal failure, with a diagnosis of “familial polycystosis”. On 23 January 2023, the patient underwent living donor kidney transplantation (mother) at Policlinico Gemelli in Rome. After transplantation, she started immunosuppressive and steroid treatment with Tacrolimus and Bactrim. Before transplantation, the patient’s serological data showed only IgG positivity for CMV (103 UA/ml) with undetected DNA, suggesting previous infection. Two months after transplantation, on 16 March 2023, the patient showed negative IgM and IgG >180 UA/ml with positivity for CMV DNA (22,992 copies/ml) (Figure 3). The patient then discontinued immunosuppressive therapy and started treatment with Valganciclovir 450 mg 1 capsule twice daily. On April 11 she attended a follow-up visit at the Transplant Outpatient Clinic with a clinical-radiological-laboratory picture suggesting a lower respiratory tract infection. After two days, she presented to the Nephrology OU with suspected *H. influenzae* pneumonia treated with levofloxacin 500 mg every 24 hours, with a Protein C Reactive (PCR) value of 88 and negative procalcitonin (PCT). During hospitalization, antibiotic therapy was continued by oral administration following IgM positivity for *Chlamydophila pneumoniae*, resulting in a marked improvement in thoracic objectivity and respiratory symptoms, with a reduction in inflammatory indices and normalization of the leukocyte formula. At the third negative determination of CMV DNA on plasma, in agreement with the referring Transplant Center, Valganciclovir therapy was discontinued. The patient is voluntarily discharged in good clinical condition, afebrile and eupneic, with advice to continue infectious evaluation at the referral clinic and continue nephrologic follow-up (Table 2).

3.3 Clinical case 3: CMV reactivation in lung transplant recipient

A 29-year-old Caucasian man is admitted to the thoracic surgical unit in Padua for a double lung transplant on April 30, 2023. The diagnosis revealed Langerhans cell histiocytosis. The patient’s medical history includes pulmonary emphysema and

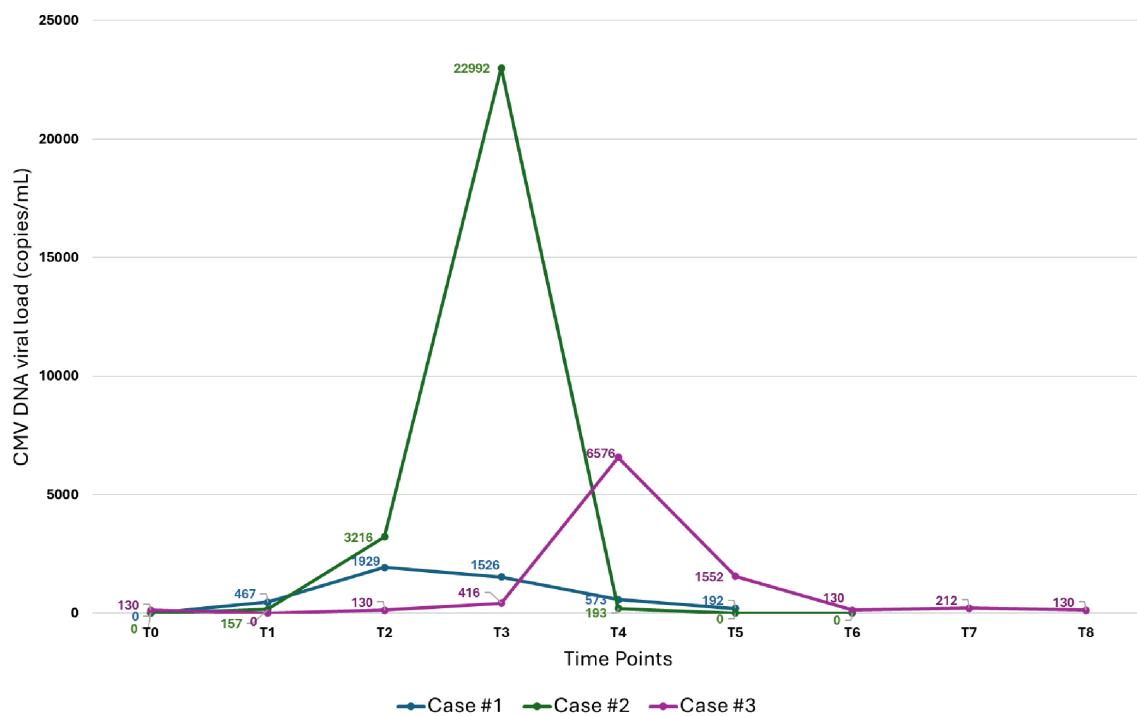


FIGURE 3
Timeline of CMV DNA viral loads during hospitalization of three patients.

idiopathic pulmonary fibrosis. After transplantation, the patient started on tacrolimus 3 mg 1 cp x 2/day as an immunosuppressant. During the hospital stay, positive blood cultures for *S. epidermidis*, *K. oxytoca* in the presence of vascular abscesses and CMV-DNA replication were reported. Previous administration of ganciclovir was replaced with oral prophylaxis of valganciclovir 450mg 2cp x 2/day. Rejection monitoring TB biopsies were performed on 6 June and found to be absent. On June 28, 2023, CMV DNAemia was under 130 copies/ml. Bronchial aspirate was positive for *Escherichia coli* and persisted for several months. On July 26, 2023, at the last check-up at the hospital in Padua, Cytomegactect 2000 IU was administered. Immunoglobulin administration was recommended for one year. The patient was admitted to the pneumology operating unit of the Renato Dulbecco hospital in Catanzaro as an outpatient. After two weeks, CMV DNA was not detectable, while serology was: anti-CMV-IgG of 42 UA/mL, anti-CMV-IgM of 25 UA/mL. After one month, on August 30, 2023, the patient was compatible with reactive CMV infection (anti-CMV-IgG: 44 UA/mL, anti-CMV-IgM: 26 UA/mL), confirmed by PCR with detectable CMV viremia (6576 copies/mL). Additionally, sputum was positive for *Aspergillus niger* and became negative after 4 months. After 5 months of follow-up, viremia decreased (<130 copies/ml), and the bronchial aspirate was negative. On March 7, 2024, DNAemia was 212 copies/mL (anti-CMV-IgG: 60 UA/mL, anti-CMV-IgM: 49 UA/mL) (Figure 3). The patient is still under control in the pneumology unit (Table 2).

4 Discussion

The vaccine for CMV infection was classified as a high priority due to the consequences of congenital infection and disease severity in immunocompromised subjects, the emergence of resistance, and the side effects of drugs, which limited the use of antiviral therapies (6, 11). In this paper, we referred to three cases of immunocompromised patients. In the first case, we illustrated the multifaceted consequences of CMV reactivation in an HIV-positive patient with poor ART adherence, contributing to persistent gastrointestinal symptoms, systemic infections, and treatment challenges. CMV is a common virus that frequently establishes latency and reactivates in immunocompromised hosts (62). CMV has a linear double-stranded DNA (dsDNA) containing 236 kb of information and more than 750 open reading frames (ORFs) packaged inside an icosahedral nucleocapsid, a large layer of tegument proteins, and an envelope containing glycoprotein complexes (20). The genome is divided into two large domains called long (L) and short (S), each of which is constituted by the central unique (UC), unique long (UL), and unique short (US) regions (21). Several CMV genotypes were defined, according to the distribution of polymorphisms along the viral genome (22). Considering the variability of gB (gB1, gB2, gB3, gB4, gB5, gB6, gB7) and gN (gN1, gN2, gN3a, gN3b, gN4a, gN4b, gN4c), it is possible to distinguish seven genotypes for both glycoproteins. The gH encoded by the UL75 gene is also able to identify two different genotypes (gH1 and gH2). Polymorphic genes

TABLE 2 Demographic and clinical characteristics of patients with CMV reactivation.

Patient Parameter	Case 1 - PLWH	Case 2 - kidney transplant	Case 3 - lung transplant
Age	36	25	29
Sex	M	F	M
Primary Diagnosis	HIV infection	Family polycystic kidney disease	Langerhans cell histiocytosis
Comorbidities	Prostatitis, anorexia-related malnutrition	COPD, chronic pancreatitis, retinitis pigmentosa	Emphysema, idiopathic pulmonary fibrosis
Type of Transplant	None	Living-donor kidney transplant	Bilateral lung transplant
Immunosuppression	suboptimal ART adherence	Tacrolimus and Corticosteroids	Tacrolimus
Coinfections	Candidemia; NDM-producing <i>K. pneumoniae</i> bloodstream infection	<i>Haemophilus influenzae</i> pneumonia; <i>C. pneumoniae</i>	<i>Aspergillus niger</i> in sputum
CMV Serostatus (Pre-transplant)	None	IgG positive; CMV DNA negative	Not Available
Initial CMV DNA load	1,929 copies/mL	22,992 copies/mL	6,576 copies/mL
Antiviral Therapy	Ganciclovir 500 mg/die x 3 weeks	Valganciclovir 450 mg BID	Ganciclovir, Valganciclovir and Immunoglobulins
Duration of Treatment	21 days	1 month	5 months
Virological Response	192 copies/mL	CMV DNA not detected	212 copies/mL
Follow-up	Partial virological response	Virological response	Partial virological response

are usually analyzed to evaluate the prevalence of viral genotype circulation among humans (2, 20, 21, 23). To determine the entry spot of the virions, the glycoproteins of the CMV envelope combine into two different complexes based on the host cellular type. The first one is composed by a trimer formed by a glycoprotein H (gH), glycoprotein L (gL), and glycoprotein O (gO) complex that binds to platelet-derived growth factor receptor α (PDGFR α) to induce pH-independent entry into the fibroblasts (24). The gN is an extensively glycosylated envelope type I glycoprotein. It is a component of the gM/gN complex that plays an essential role in CMV replication. This gM/gN complex is the most abundant protein complex in the virion envelope (23). Pentamer-mediated entry requires the presence of gO, as well as a low endosome pH. A crucial role in viral entry is played by glycoprotein B (gB) (25). The high tropism of CMV for different cell types requires the interaction between several virus-encoded glycoproteins and receptors on the host cell surface (26). The CMV gB is encoded by the UL55 gene and is synthesized as a polypeptide composed of about 900 amino acids that consists of 5 structural domains (I-V) and undergoes cleavage by furin (26). To infect epithelial, endothelial, and likely many other cell types, the gH, gL, UL128, UL130, and UL131A pentamer can bind to Olfactory Receptor family 14 subfamily I Member 1 (OR14I1) or Neuropilin 2 (NRP2) on their cell surface, inducing virion endocytosis (Figure 4) (27).

Among PLWH, CMV seropositivity has been associated with increased epithelial gut damage, microbial translocation, and systemic inflammation, even in individuals receiving long-term ART. These processes are hypothesized to contribute to chronic immune activation and the development of non-AIDS

comorbidities (63–65). In the present case, CMV reactivation was identified through the detection of CMV DNAemia, coinciding with exacerbation of gastrointestinal symptoms and chronic colonic inflammation. These findings align with recent evidence demonstrating that CMV replication is a significant contributor to gut permeability and the subsequent translocation of microbes into the circulation, leading to systemic inflammation. CMV replication in the gastrointestinal tract can compromise intestinal barrier integrity by disrupting tight junctions of polarized intestinal cells and enhancing transepithelial permeability, contributing to microbial translocation in PLWH patients (65–67). Studies have shown that CMV coinfection and reactivation are associated with elevated markers of gut damage, such as intestinal fatty acid-binding protein (I-FABP), and microbial translocation markers, including lipopolysaccharide (LPS) and (1 $\rightarrow$ 3)- β -d-glucan (BDG) (63). Complications such as candidemia and NDM resistance phenotype *K. pneumoniae* bloodstream infections highlight the susceptibility of this patient to opportunistic infections. Persistent microbial translocation, secondary to CMV-associated gut damage, may have contributed to these bloodstream infections (63, 68, 69). The patient's poor adherence to ART further complicated his clinical course, leading to intermittent virological failure and suboptimal immune reconstitution (70). The successful reduction of CMV DNAemia following ganciclovir therapy underscores the potential benefits of targeted antiviral strategies. However, the recurrent nature of CMV reactivation and its long-term impact on immune health necessitate preventive measures beyond pharmacological management (71). The development and deployment of an effective CMV vaccine could represent a

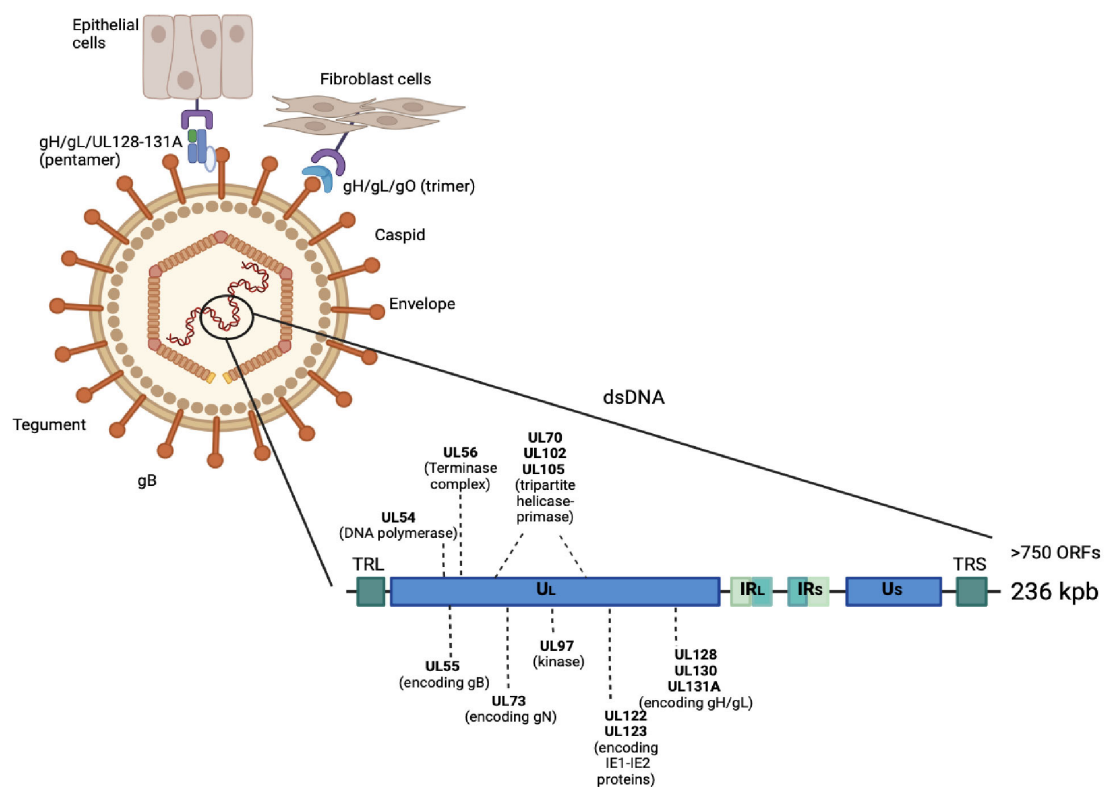


FIGURE 4
CMV viral structure and host-cell interaction, including genome organization.

transformative advancement in the care of PLWH (11). By preventing CMV infection and reactivation, such a vaccine would reduce epithelial gut damage, mitigate chronic immune activation, and ultimately decrease the incidence of non-AIDS comorbidities (71). In particular, a safe CMV vaccine would be crucial for individuals with poor ART adherence or compromised immune status, as they remain at high risk for CMV-related complications. In people with compromised immune systems, such as SOT patient (case 2), where patients undergo pharmacological immunosuppression to prevent rejection, CMV has the potential to cause severe disease. Because of these adverse effects, CMV prevention is part of the standard of care (72). This can be accomplished through antiviral prophylaxis with Valganciclovir for at least 3 months after SOT. Or as a preventive therapy, which involves the administration of antiviral drugs only after CMV replication is detected. With this strategy, CMV nucleic acid amplification testing monitors patients regularly at close time points during the first 3 months after SOT. If CMV DNA is detected, antiviral drugs such as Valganciclovir are administered to treat patients until the virus is no longer detectable in the blood (73). Immune dysregulation caused by immunosuppressive drugs and CMV reactivation may have favored or facilitated a possible interaction with secondary *C. pneumoniae* infection, potentially contributing to the severity of respiratory symptoms. This interpretation is consistent with clinical observations that CMV reactivation in transplant and critically ill patients is often

accompanied by bacterial and fungal co-infections and with experimental data showing enhanced pro-inflammatory responses during certain viral-bacterial co-infections, which may amplify lung injury and impair pathogen clearance (74–76). Sustained exposure to immunosuppressive agents may predispose patients to subsequent CMV reactivation, despite prior effective antiviral therapy. Moreover, prolonged use of antiviral drugs, such as Valganciclovir, may have side effects such as neutropenia, gastrointestinal problems, and renal toxicity. Therefore, vaccination in patients with a compromised immune system would be considered necessary. Finally, Lung transplantation (LTx) is the definitive treatment option for patients with severe pulmonary diseases (case 3) (77). Cytomegalovirus is one of the most prevalent viral pathogens contributing to morbidity after SOT (78). In immunosuppressed patients, CMV infection can be asymptomatic but more frequently presents as CMV syndrome, characterized by fever, malaise, leukopenia, thrombocytopenia, and elevated serum transaminases, or as organ-specific CMV diseases, such as pneumonia, gastroenteritis, or hepatitis (79). Transplant-associated CMV infection may occur as a primary infection in seronegative recipients, as a reactivation of latent CMV, or because of reinfection with a new CMV strain (80). Due to its immunomodulatory effects, CMV infection may increase susceptibility to other opportunistic infections, such as *Aspergillus* species, *Pneumocystis jirovecii*, *Nocardia*, and Epstein-Barr virus, which could contribute to the overall burden of infection-related

complications (81–83). Further, lung transplant recipients are particularly at risk for severe infections from common community-acquired respiratory viruses: Respiratory syncytial virus (RSV), Parainfluenza virus (PIV), Rhinovirus, Coronavirus, Human Metapneumovirus, Influenza, and Enterovirus (84). The early identification and management of CMV viremia have been shown to improve clinical outcomes, underscoring the need for integrated monitoring strategies targeting both viral and fungal pathogens (82, 83). The treatment of symptomatic cases involves a combination of antiviral therapy and immunosuppression reduction. Both valganciclovir and ganciclovir are effective in treating symptomatic CMV disease (81). In CMV infections, intravenous immunoglobulin (IVIG) is administered for the treatment or prevention (85). However, vaccination of recipients significantly reduced severe symptoms and the risk of graft rejection (86). Although acquired immunity does not consistently prevent reinfection (58).

Reinfection and mixed events are directly related to CMV genome high variability, which can facilitate immune evasion, increase viral replication, and decrease antiviral efficacy. CMV exhibits recombination events, among other evolutionary phenomena, due to the multitude of infections (2). High level of within-host CMV strains diversity is not related to mutational rates, but rather to frequent mixed infections (about 61%) identified using serial and different samples, particularly in immunocompromised subjects. Infection of different viral strains determined recombination events, regarded as putative drivers of CMV evolution, influencing its pathogenesis by promoting viral cell entry (87). The interaction between host and virus generates a positive selection in specific CMV genomic regions, such as surface glycoproteins, avoiding immune recognition (3). The gB and gH/gL proteins elicit serum neutralizing antibodies, blocking entry into target cells (88). Following natural infection, gH-specific neutralizing antibodies were detected in convalescent serum samples and were initially considered for the treatment and prophylaxis of infections. Recently, the strain-specific neutralization capacity of gH antibodies has been demonstrated. Considering three monoclonal antibodies (2B10, 6E3, 3C11), two were effective at blocking distinct CMV strains, while 2B10 was strictly strain-specific against a single residue on the gH surface. This underlines the importance of protective immunity against gH polymorphic sites (88). The strain-specific serological methods could be useful in determining CMV strain diversity, multiple infections, and reinfection, thus identifying the appearance of new antibodies over time against the antigenic determinants on gH and gB envelope glycoproteins (89). CMV genotypes can be identical in different geographic areas, although new/rare viral strains can be detected in restricted areas of the world or in specific risk groups: gB2 was foremost present among PLWH and children with congenital infection, gB1 was the most prevalent among SOT recipients and immunocompromised patients, and finally, gB4 was found in newborns with sepsis-like syndrome (90). CMV genomic evolution occurred by genetic drift within geographically distinct populations. The CMV genome variations interacted to

cause geographical differences and their spread to specific areas (91). Interestingly, the CMV population appeared to be relatively stable within tissue compartments in a single host, while it rapidly evolved during the colonization of different compartments. Each compartment reportedly exhibited a unique selective pressure, mutation, or polymorphism on specific genes that can affect the tropism of the viral population, increasing its fitness (92). The analysis, performed on the UL55, UL73, UL75, US28, and UL144 genomic regions in urine, saliva, and plasma samples, showed the presence of multiple genotypes in primary infection. The detection of several CMV viral strains in different specimens from the same subject was common (10/15) (57). In this study, 10 subjects had gN2 only in saliva, four subjects had unique gB genotypes found in saliva or urine, while different gH types were detected in different samples, and one subject displayed a unique US28 genotype only in saliva. New genotypes within the same specimen were observed with a median time of 5 months. The frequency of each genotype was probably related to transmission variability or infection reactivation (57). Hypothetically, the different genotypes of CMV may influence its virulence, though its impact on disease severity is still debated, and there are conflicting results (93). Pathogenesis may be directly dependent on tissue tropism and the local site of replication. In PLWH and immunocompromised patients, the frequency of gB2 in patients with retinitis compared to those without was not higher. On the contrary, a higher frequency of gB4 was found in semen samples from the genitourinary tract: this tissue specificity was not revealed for gB1, gB2, and gB3 (59). However, the extensively reported possibility of mixed infection within a patient underlined the difficulty in studying the association between pathogenesis and a particular genotype, such as potential virulence or symptomatic disease (59). Genetic variation could affect clinical outcome, especially in immunocompromised people. In patients receiving marrow transplants, complications of neutropenia and subsequently death appeared significantly associated with gB3 and gB4, possibly because they escape immune recognition and persist in the marrow, causing more damage (94). The increased susceptibility to reinfection was related to the recognition of gB structural differences by antibodies, suggesting CMV variants and naturally occurring mutations as responsible for a less effective immune response (95). The sequencing techniques highlighted the compartmentalization of gB epitopes, even if the major viral populations were identical between host anatomic compartments. The presence of low-frequency viral variants could explain the partial efficacy of vaccines (96). GB-specific antibodies limited the dissemination of viruses between tissue compartments, selecting the more advantageous CMV strains in each compartment and the subsequent formation of genetically distinct viral variants (96). Mixed CMV infection and viral strain compartmentalization (cervical and breast milk) were also identified in HIV-infected women. In this situation, the congenitally transmitted virus was the one with the highest abundance in the cervix and was genetically distinct from the breast milk strains (97). The gB/MF59 vaccine, constituted with the Towne gB1 genotype strain, showed a lack of protection in vaccine recipients infected with gB1

within 3 months of primary infection. Strain-specific immunity was suggested as being protective against CMV infection (57). Recombinant viral strains are usually positively selected and occur between superinfecting as well as reactivating genomes. Studies based on the analysis of reconstructing haplotypes from short-read sequenced data showed that the most parsimonious explanation of high within-host CMV diversity is the frequent presence of mixed infections, whereas CMV in non-mixed infections is no more diverse than other DNA viruses (58). The availability of CMV sequences from around the world can be used to evaluate viral antigens or gB-specific strains to induce cross-protection against globally circulating variants (91). Evolutionary phenomena during CMV infection, such as multiple CMV strains coinfection and recombination events, were related to viral pathogenesis and to the complexity of developing an effective vaccine.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of the Calabria Region (#357, approved on December 20, 2024). Written informed consent for participation was not required for these case series because all patient data were provided in an anonymized form, and no identifiable information was included. The laboratory data were produced from routine diagnostic procedures. On human samples, no additional tests were performed for research purposes. Accordingly, the study did not involve any intervention beyond standard clinical care.

Author contributions

NM: Visualization, Writing – original draft, Conceptualization. GP: Visualization, Writing – original draft. CM: Writing – original

draft. MP: Writing – original draft. EG: Writing – original draft, Data curation. MM: Writing – original draft, Data curation. SG: Writing – original draft, Data curation. GB: Methodology, Writing – original draft. CP: Methodology, Writing – original draft. GM: Supervision, Writing – review & editing. AQ: Visualization, Conceptualization, Writing – review & editing.

Funding

The author(s) declare that no financial support was received for the research and/or publication of this article.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

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