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Special Issue Reprint

Viral Infections in Immunocompromised Hosts

Edited by
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This is a reprint of the Special Issue, published open access by the journal *Viruses* (ISSN 1999-4915), freely accessible at: https://www.mdpi.com/journal/viruses/special_issues/03V5644L36.

For citation purposes, cite each article independently as indicated on the article page online and as indicated below:

Lastname, A.A.; Lastname, B.B. Article Title. *Journal Name* **Year**, *Volume Number*, Page Range.

ISBN 978-3-7258-4529-3 (Hbk)
ISBN 978-3-7258-4530-9 (PDF)
https://doi.org/10.3390/books978-3-7258-4530-9

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Editorial

The Evolving Landscape of Viral Infections in Immunocompromised Hosts

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There has arguably never been a more dynamic and challenging time to be a clinician or researcher in the field of transplant and immunocompromised infectious diseases. Advances in immunosuppressive therapies, transplantation medicine, and cellular therapies have transformed outcomes for patients with hematologic or solid malignancies and autoimmune diseases, as well as those with hematopoietic stem cell or solid organ transplants. However, these advancements come with an evolving spectrum of viral infections that demand our continued vigilance and innovation in diagnostics, treatment, and prevention strategies. The rise of chimeric antigen receptor (CAR) T-cell therapy and bispecific antibodies has also introduced novel infection complications, including herpesvirus reactivations, and underscores the need for updated surveillance and prophylactic strategies.

Despite overall improvements in infection-related mortality, viral infections remain a major cause of morbidity and excess healthcare costs in immunocompromised patients. Reactivation of latent viruses, such as herpesviruses and polyomaviruses, along with episodic infections from respiratory and gastrointestinal viruses, presents persistent challenges, while emerging infections, like those from arboviruses, further complicate the clinical picture. The use of advanced molecular diagnostics has improved our ability to detect viral reactivation early but also introduces challenges in interpretation, particularly when low-level viremia or DNAemia does not correlate with disease. In addition, antiviral resistance is increasingly reported, particularly with prolonged prophylaxis or treatment in transplant recipients, necessitating the development and evaluation of newer agents.

In this Special Issue of *Viruses*, we bring together a multidisciplinary group of experts to explore the latest developments in the prevention, diagnosis, and management of viral infections in immunocompromised hosts. Several key themes emerge from the contributions within this issue:

Optimizing and Expanding Antiviral Therapies: A comparative study evaluating maribavir and foscarnet for CMV treatment in transplant recipients highlights the importance of balancing efficacy with toxicity, particularly nephrotoxicity. Another study investigates the impact of CMV infection on renal function in liver transplant recipients, emphasizing that viral infection may exacerbate chronic kidney disease and negatively affect long-term graft survival. Additionally, a review of adenovirus infection in transplant recipients outlines current and emerging antiviral strategies, including brincidofovir and adoptive T-cell therapy. These findings underscore the need for early diagnosis, individualized treatment, and continued innovation in antiviral drug development for high-risk viral pathogens.

Expanding the Scope of Antiviral Stewardship: A comprehensive review outlines principles of antiviral stewardship, focusing on opportunities to individualize therapy and

reduce unnecessary antiviral use. Stewardship interventions are particularly critical in managing CMV, EBV, and respiratory viruses in transplant recipients to prevent toxicity, resistance, and excess healthcare costs.

Emerging Challenges with Cellular Therapies and Transplant Inflammation: A focused review of herpesvirus infections following CAR T-cell therapy and bispecific antibodies details reactivation risks, particularly for HSV, VZV, and CMV, and highlights the importance of antiviral prophylaxis and the limitations of current surveillance approaches in this population. Separately, a study of cytokine dynamics in solid organ transplant recipients explores how the post-transplant inflammatory milieu influences herpesvirus reactivations. These insights suggest a need for integrated immune monitoring to anticipate and manage viral complications across different transplant modalities.

CMV in Non-Transplant Immunosuppressed Hosts: A detailed review of CMV colitis in inflammatory bowel disease (IBD) patients underscores the diagnostic and therapeutic challenges in differentiating between CMV disease and IBD flares. The role of tissue pathology and immunohistochemistry is emphasized, as well as antiviral strategies for steroid-refractory ulcerative colitis.

Innovations in Diagnostics: Novel biomarkers such as CMV cell-mediated immunity assays and viral metagenomics are discussed across several studies, suggesting a future in which risk stratification and treatment decisions will be increasingly personalized.

Viral Infections and Transplant Outcomes: Arbovirus transmission through transplantation, alongside the impact of respiratory viral infections on lung transplant allograft rejection and donor-specific antibody formation, is examined. A comprehensive review also highlights the risks of hepatitis B virus reactivation in liver transplant recipients. Together, these studies underscore the need for integrated infection control strategies, careful donor screening, and vigilant post-transplant monitoring.

The research presented in this Special Issue reflects the dedication and collaborative efforts of experts in virology, immunology, gastroenterology, and transplant medicine. Their work not only deepens our understanding of viral infections in immunocompromised patients but also highlights the urgency of refining diagnostic thresholds, validating new treatment options, and implementing patient-centered stewardship strategies. We extend our sincere gratitude to the authors for their valuable contributions, to the editorial team at *Viruses* for supporting this initiative, and most importantly to the patients and their caregivers, whose experiences fuel our commitment to advancing the science of viral infection management in vulnerable populations.

Conflicts of Interest: The authors declare no conflict of interest.

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Article

Real-World Comparison of Maribavir to Foscarnet for the Treatment of Cytomegalovirus in Solid Organ and Hematopoietic Stem Cell Transplant Recipients

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Abstract: Cytomegalovirus (CMV) infection in solid organ transplant (SOT) and hematopoietic cell transplant (HCT) recipients may increase the risk of rejection or allograft dysfunction, other infection(s), and morbidity and mortality. Treatment can be challenging due to medication-associated toxicities. Maribavir (MBV) is a promising option for the treatment of resistant or refractory (R/R) CMV infection in lieu of foscarnet (FOS), which has long been the recommended therapy for (val)ganciclovir-resistant infection. This was a single-center retrospective study of clinical outcomes of patients who received MBV compared to a control group who received FOS for an episode of CMV infection. Each cohort consisted of 27 episodes of CMV infection. Twenty patients in the MBV cohort and from the FOS cohort cleared the infection, with five and three patients developing MBV or FOS resistance, respectively. There were no statistically significant differences in failure of therapy as evidenced by persistent DNAemia ($p = 0.56$) or development of antiviral resistance ($p = 0.24$). In conclusion, MBV was as effective as FOS for the treatment of R/R CMV infection and was better tolerated without increased risk of antiviral resistance.

Keywords: CMV; resistance; maribavir; foscarnet; immunocompromised

1. Introduction

Despite currently available antiviral therapy, cytomegalovirus (CMV) remains a challenging opportunistic infection to treat, with significant morbidity and mortality in solid organ transplant (SOT) and hematopoietic cell transplant (HCT) recipients. First line antiviral agents, valganciclovir (VGC) and ganciclovir (GCV), are myelotoxic [1]. Other antiviral options, such as foscarnet (FOS) and cidofovir (CDV), have multiple toxicities, including nephrotoxicity, and typically require administration with close monitoring in an inpatient setting [2]. Even with effective dosing for prophylaxis and treatment, prolonged exposure to antiviral medications and incomplete suppression of CMV may contribute to the development of antiviral resistance mutations [3].

Refractory or resistant (R/R) CMV infection, which can occur independently or concurrently, confers worse clinical outcomes, from increased rates of rejection, allograft failure, hospitalizations, and mortality [4–7]. Resistant CMV infection refers to the detection of genetic mutation (s) that predict decreased susceptibility to antiviral therapy, whereas refractory CMV infection is defined by the persistence of symptoms or increased viral load by one log10 after two weeks of appropriately dosed therapy [8,9]. Risk factors for R/R CMV infection include T-cell depletion, lack of CMV-specific immunity, intense immunosuppressive therapy, poor absorption of antiviral therapy, sub-optimal antiviral

dosing, prolonged antiviral exposure, prior antiviral exposure, high CMV viral load, and intermittent low-level CMV DNAemia [8].

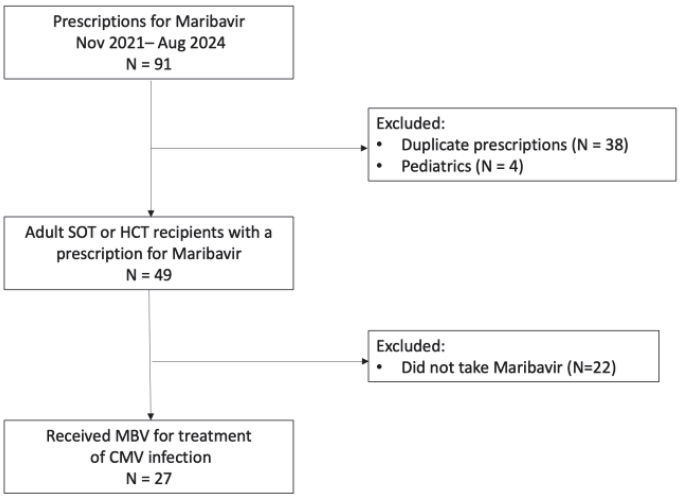
Maribavir (MBV) is a novel agent with demonstrated efficacy in achieving clearance of DNAemia in R/R CMV infection without the myelotoxicity associated with VGC or GCV or the nephrotoxicity associated with FOS or CDV [10,11]. Prior to the introduction of MBV, FOS had been the recommended therapy for resistant CMV or for those who may not tolerate GCV or VGC [1,5]. A phase III clinical trial demonstrated superior efficacy of MBV achieving viral clearance in R/R CMV infection compared with investigator-assigned therapy after 8 weeks of treatment in SOT and HCT recipients and was maintained for 4 weeks post-completion of therapy [12]. However, there are less data on the real-world efficacy of MBV, especially in high-level CMV DNAemia. A few small studies have reviewed the utility of MBV, but there have been no direct comparisons with other antiviral agents for R/R CMV infection [13,14].

To address this question, we evaluated the clinical outcomes of SOT and HCT recipients at a high-volume transplant center to determine the effectiveness and safety of MBV compared to a control group who received FOS for CMV infection.

2. Materials and Methods

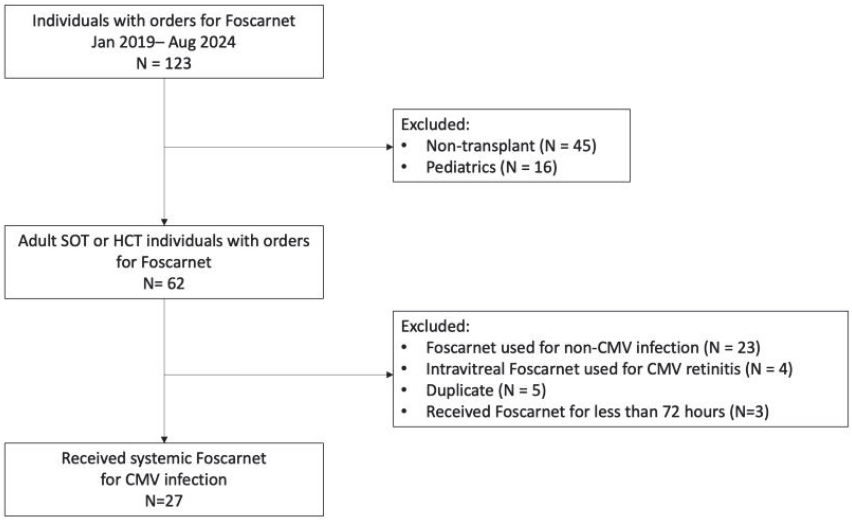
This was a single-center retrospective cohort study of SOT and HCT recipients who received MBV for the treatment of CMV infection at the University of California, Los Angeles (UCLA) Health Centers. To identify an appropriate comparison control group, we queried our electronic medical record system for patients who received FOS for an episode of CMV infection. The study was approved by the Institutional Review Board.

Two internal databases of individuals with prescriptions or orders for MBV from 1 November 2021 to 31 August 2024 and FOS from 1 January 2019 to 31 August 2024 were reviewed. In both groups, pediatric patients (under age 18), duplicate orders or prescriptions, and those who received therapy for less than 72 h were excluded. From the MBV group, patients who were prescribed MBV but did not actually take MBV were excluded. From the FOS group, non-transplant recipients, individuals who received FOS for a non-CMV infection, and individuals who did not receive systemic FOS were excluded (Figure 1a,b).



(**a**)

Figure 1. *Cont*.



(**b**)

Figure 1. (**a**). Flow diagram of exclusion criteria for the maribavir cohort. (**b**). Flow diagram of exclusion criteria for foscarnet cohort.

Electronic medical records were reviewed, and data collected included demographic information, type of transplant, immunosuppression (IS), antiviral prophylaxis, peak CMV viral load, CMV T-cell immunity panels, CMV genotypic resistance testing, antiviral treatment, antiviral adverse effects, co-infection(s), and mortality. Bloodwork focusing on absolute neutrophil count (ANC), absolute lymphocyte count (ALC), renal function, and electrolytes (potassium, calcium, magnesium, and phosphorous) was also compiled from time of initiation, switch, and completion of CMV therapy. Renal dysfunction was defined as a 25% or greater change in glomerular filtration rate. The collected characteristics and outcomes were based on episode of CMV infection, with the exception of mortality. For individuals with multiple episodes of CMV infection, only the final episode of infection was counted in the mortality analysis.

Typical post-transplant prophylaxis regimens have been described elsewhere, and all SOT recipients received CMV prophylaxis corresponding to the organ(s) transplanted and donor/recipient (D/R) serostatus risk based on American Society of Transplantation (AST) and international guidelines [1,15,16]. For SOT recipients, risk was defined as high risk (D+/R-), intermediate risk (R+), and low risk (D-/R-). For HCT recipients, high risk was defined as R+. Duration of prophylaxis varied with the type of transplanted organ from 3 months to life-long based on risk. All CMV seropositive HCT recipients received CMV prophylaxis until day +100 in accordance with internal protocols. Any adjustment in prophylaxis and use of alternative agents were made at the discretion of an Infectious Diseases physician or primary transplant physician. MBV prescriptions were for 400 mg orally twice daily. FOS was dosed at 90 mg/kg intravenously every 12 h and adjusted for renal function by an Infectious Diseases physician.

Asymptomatic CMV DNAemia was defined as the detection of CMV DNA in plasma using quantitative polymerase chain reaction (PCR) alone [1,17]. CMV disease was defined as either proven, probable, or possible end-organ disease based on the 2024 consensus definitions of CMV infection and disease in transplant patients [17]. Successful treatment response was defined as the resolution of any presenting symptoms and clearance of DNAemia with one quantitative plasma CMV PCR test that was either negative or detectable but below the level of quantification. Relapse or recurrent DNAemia or disease was defined as a positive plasma CMV PCR with or without symptoms occurring within 8 weeks of stopping MBV or FOS. Resistant CMV was defined as the detection of gene mutations resulting in diminished susceptibility to an antiviral [8].

All CMV quantitative PCR testing was performed at the UCLA Clinical Microbiology Laboratory. Testing was performed using Roche AmpliPrep CMV PCR assay. In October 2023, CMV quantitative PCR testing switched to Roche Cobas 6800/880 CMV PCR assay with higher analytical sensitivity and the level of quantification changed from <137 IU/mL to <35 IU/mL. CMV drug resistance testing was performed at reference laboratories, ARUP or Eurofins Viracor. CMV T-cell immunity assays were performed via intracellular cytokine staining by flow cytometry (Viracor Eurofins CMV inSIGHT™ T Cell Immunity Testing). Statistical analysis was performed on JMP Pro-17. Numeric variables were analyzed using a nonparametric approach. Categorical variables were analyzed by an unpaired two-sample Wilcoxon test. Statistical significance was defined as $p < 0.05$. Given the small size of the cohort studied, a multivariate analysis was not performed, as it could lead to overfitting of the statistical models.

3. Results

3.1. Demographic and Clinical Characteristics

Twenty-seven SOT and HCT recipients with asymptomatic CMV DNAemia or disease received MBV and were included in the final analysis (Figure 1a). Baseline demographics and characteristics were similar between the two groups (Table 1). There were no statistically significant differences between the two groups regarding age, sex, race, type of transplant, or IS. The majority of patients were on triple IS consisting of tacrolimus, mycophenolate, and prednisone.

Table 1. Characteristics and demographics of maribavir and foscarnet cohorts.

Characteristic		Maribavir (*n* = 27)	Foscarnet (*n* = 27)	*p*-Value
Age, median (range)		59 (29–75)	60 (18–74)	0.80
Sex, *n* (%)	Male	13 (48.15)	16 (59.3)	0.59
	Female	14 (51.85)	11 (40.7)	
Race, *n* (%)	Asian	3 (11.11)	3 (11.1)	0.65
	Black or African-American	2 (7.41)	4 (14.8)	
	White	10 (37.04)	12 (44.4)	
	Hispanic or Latino	12 (44.44)	8 (29.6)	
Type of Transplant, *n* (%)	SOT	19 (70.37)	22 (81.5)	0.53
	HCT	8 (29.63)	5 (18.5)	
	Heart	1 (3.70)	3 (11.1)	
	Lung	9 (33.33)	13 (48.1)	
	Liver	2 (7.41)	0 (0)	
	Kidney	7 (25.93)	6 (22.2)	
	alloHCT	7 (25.93)	5 (18.5)	
	autoHCT	1 (3.70)	0 (0)	
Treated for rejection, GVHD, or relapsed disease, *n* (%)		3 (11.11)	2 (7.40)	0.39
Immunosuppression, *n* (%)	Four or more	1 (3.70)	0 (0)	0.95
	Triple therapy	19 (70.37)	22 (81.5)	
	Dual therapy	4 (14.81)	5 (18.5)	
	One agent	2 (7.41)	0 (0)	
CMV Serostatus Risk, *n* (%)	High or Moderate	25 (92.59)	22 (81.5)	0.22
	Low	2 (7.41)	5 (18.5)	
On CMV prophylaxis at time of episode of infection, *n* (%)		18 (62.96)	23 (85.2)	0.11
First occurrence of CMV infection, *n* (%)		16 (59.26)	11 (33.3)	0.06
Recurrent CMV infection, *n* (%)		11 (40.74)	16 (66.7)	0.17

Abbreviations: CMV, cytomegalovirus; HCT, hematopoietic cell transplant; SOT, solid organ transplant.

There were 27 unique patients in the MBV group. In the FOS group, we identified 27 episodes of FOS administration for the treatment of asymptomatic CMV DNAemia or disease (Figure 1b). Two patients had more than one episode of CMV infection that was treated with FOS. In the MBV cohort, 18/27 (63%) were on CMV prophylaxis at time of infection. The most commonly used prophylaxis was VGC (12, 66.7%), followed by letermovir (LET, 4, 22.2%) and acyclovir (ACV, 2, 11.1%). In the FOS cohort, the majority of patients (23/27, 85.2%) were on CMV prophylaxis at the time of infection. Similar to the MBV group, the most commonly used agent was VGC (16/27, 69.6%). LET (3/27, 13%) and ACV (1/27, 4.3%) were also used as prophylaxis. Three patients (13%) were on maintenance MBV at time of CMV infection.

3.2. Initial CMV Management and Assessment of Resistance

In both the MBV and FOS cohorts, the majority of patients had asymptomatic CMV DNAemia and were initially treated with GCV or VGC (Table 2). Five patients received MBV as the initial treatment due to pre-existing leukopenia or history of intolerance to first-line therapy. In contrast, 10 patients received FOS as the initial treatment. The reasons for choosing FOS as the initial therapy over VGC or GCV were due to concerns for resistant CMV, given evidence of breakthrough on prophylaxis or pre-existing cytopenia.

Table 2. Presentation of CMV infection and treatment outcomes.

Presentation and Outcomes		Maribavir (n = 27)	Foscarnet (n = 27)	p-Value
Peak viral load, median (range)		10,538 (444–250,709)	24,184 (1100–889,657)	0.49
Asymptomatic CMV DNAemia		24 (88.9)	21 (81.5)	0.27
Initial Treatment, *n* (%)	Valganciclovir	17 (63.0)	12 (44.4)	<0.0001
	Ganciclovir	5 (18.5)	5 (18.5)	
	Maribavir	5(18.5)	--	
	Foscarnet	--	10 (37)	
Number of failed regimens before switch to MBV or FOS, *n* (%)	Started with MBV or FOS	5 (18.5)	10 (37)	0.52
	Failed 1	13 (48.2)	8 (29.6)	
	Failed 2	9 (33.3)	7 (25.9)	
	Failed 3	0 (0)	2 (7.4)	
Treatment duration with MBV or FOS, median days (range)		44 (11–126)	21 (4–148)	0.01
Time until clearance of DNAemia, median days (range)		23 (2–67)	16 (4–65)	0.65
DNAemia cleared, *n* (%)		20 (74.1)	18 (66.7)	0.55
Recurrence within 8 weeks of stopping MBV (n = 20) or FOS (n = 18), *n* (%)		2 (10.0)	5 (38.9)	0.13
Developed resistance, *n* (%)		5 (18.5)	3 (11.1)	0.64

Abbreviations: CMV, cytomegalovirus; FOS, foscarnet; MBV, maribavir.

The median time to switch from initial therapy to MBV was 22 days. At the time of switch in therapy, median ANC was 1.9×10^3 cells/mL and ALC was 0.84×10^3 cells/mL. In general, patients demonstrated a trend towards receiving first-line therapy for a longer period of time before switching to FOS, with a median duration of 38 days, although this difference did not reach statistical significance ($p = 0.158$). At the time of switch in therapy, median ANC was 1.9×10^3 cells/mL and ALC was 0.68×10^3 cells/mL. T-cell immunity panels were not uniformly collected in either group but were more commonly ordered in the FOS cohort (52%) than the MBV cohort (19%) ($p = 0.002$). Of those who did have a CMV T-cell immunity panel, the majority had an immune response below 0.2%, with no significant difference in terms of CD4 or CD8 response between the MBV versus FOS patients ($p = 0.77$ and $p = 0.91$, respectively). Resistance testing was performed in 93% (25/27) of cases from the MBV cohort (Table 3). Of those, 17 (68%) were found to

have resistance, most commonly at UL97 conferring resistance to VGC/GCV. Two had resistance to LET with UL97 C325W. In the FOS cohort, resistance testing was performed in all patients, which revealed UL97 or UL54 resistance genes in 24 patients (85.2%) conferring VGC/GCV and in some cases CDV and MBV resistance. In both groups, moderate to high VGC/GCV UL97 resistance at codons 460, 520, 594, 595, and 603 was detected.

Table 3. Distribution of antiviral drug-resistance mutations arising during maribavir or foscarnet treatment.

Antiviral Treatment	Gene	Amino Acid Substitution	Predicted Antiviral Resistance	*n*
MBV	UL97	T409M	MBV	3
MBV	UL97	H411Y	MBV	2
MBV	UL97	C480F	MBV, GCV	1
FOS	UL54	G841A	FOS, CDV, GCV	1
FOS	UL54	Q578H	FOS, CDV, GCV	1
FOS	UL54	A809V	FOS	1

3.3. Virologic and Clinical Response to MBV and FOS

Twenty patients in the MBV cohort had resolution of infection, with two experiencing recurrence as measured by CMV DNAemia within 8 weeks of stopping therapy (Table 2). Seven patients were continued on a longer course of MBV as either suppression or prophylaxis, with two developing breakthrough CMV DNAemia. Median duration to clearance was 23 days, but patients remained on therapy for a median time of 44 days.

Patients received FOS for a median of 21 days, with 66.7% (18/27) clearing infection in a median time of 16 days (Table 2). Five had recurrence of DNAemia within 8 weeks of stopping therapy. The time of FOS administration was shorter than the time receiving MBV ($p = 0.01$), which was largely related to issues regarding the need for intravenous administration and concern for toxicity. There was no significant difference in failure defined as persistent DNAemia by viral load at the time of the switch in patients receiving either MBV ($p = 0.68$) or FOS ($p = 0.27$).

In the comparison analysis, there was no statistically significant difference in drug failure as evidenced by persistent DNAemia ($p = 0.56$). In the MBV cohort, eight patients had repeat resistance testing (one due to the development of breakthrough infection while on secondary prophylaxis and seven due to the inability to clear DNAemia, Table 3). Five were found to have MBV resistance and were switched to FOS; of these five patients, four demonstrated virologic clearance and one died. In the FOS cohort, six patients had repeat resistance testing (three due to refractory asymptomatic CMV DNAemia and three due to refractory end-organ disease). Of the six patients, three had developed resistance to FOS (two with end-organ disease and one with asymptomatic CMV DNAemia). MBV resistance occurred at UL97 T409M, H411Y, and C480F. FOS resistance was identified at UL54 A809V, G841A, and Q578H.

There was no difference in the risk of developing resistance to therapy while receiving MBV or FOS ($p = 0.64$). These cases of resistance to FOS occurred prior to the introduction of MBV in the market, so one case was enrolled in a clinical trial, one was switched to CDV+GCV, and one had no change in therapy due to mortality. Three patients were switched from FOS to MBV in order to avoid intravenous therapy or potential side effects.

3.4. Adverse Effects

In terms of adverse effects, seven patients (25.9%) reported dysgeusia with MBV. Eight patients on FOS reported adverse effects (8/27, 29.6%), which included nausea (four), headaches (two), and genital ulcers (two). Strikingly, twenty-three FOS patients, (85.2%) compared with zero MBV patients, experienced either electrolyte imbalances or

renal dysfunction ($p < 0.001$). Two FOS patients required a switch or hold in therapy due to renal dysfunction.

3.5. Mortality

All-cause mortality occurred in six patients (22.2%) from the MBV cohort. One fatality was suspected to be due to complications of post-transplant lymphoproliferative disease and R/R CMV disease with colitis and gastrointestinal bleeding.

All-cause mortality was observed after eight episodes of CMV (29.6%) from the FOS group; in four of those, complications of CMV disease were thought to be a contributing factor. Complications were mostly related to end-organ disease, such as gastrointestinal bleeding with CMV colitis or respiratory failure with CMV pneumonia and co-infection. There was no significant difference in all-cause mortality between groups ($p = 0.14$).

4. Discussion

The 2021 FDA approval of MBV added another therapeutic option for the treatment of CMV. MBV has the potential advantages of not causing myelotoxicity or nephrotoxicity, is available as an oral formulation, and does not require inpatient administration or frequent lab monitoring. Despite multiple treatment options, CMV infection remains a common and sometimes challenging complication amongst SOT and HCT recipients. A criticism of the SOLSTICE study was the concern for external validity with using MBV for the treatment of R/R CMV [18]. Using a real-world cohort, we compared and evaluated treatment outcomes for CMV infection using MBV or FOS.

To the best of our knowledge, this is the largest real-world cohort of MBV in comparison to conventional therapy with FOS. In this study, MBV appeared to be as effective but better tolerated than FOS. Although we were not able to show superiority as seen in the SOLSTICE study, MBV remains a viable treatment option in this real-world analysis and there were no statistically significant differences in rates of treatment failure [12]. Unlike the SOLSTICE study, where subjects were treated with MBV for 8 weeks, it is common practice at our institution to treat CMV infection until resolution of clinical symptoms and clearance of DNAemia with one negative (or below the level of detection) plasma CMV PCR. We found that patients were treated for a median time of 44 days with MBV and had a median time to clearance of 20 days. There was also a lower rate of relapse in the MBV group compared to the FOS group within 8 weeks of stopping therapy. This may be due to the receipt of extended MBV therapy in patients deemed to be at risk for relapse with shorter courses of therapy. Alternatively, this observation may be explained by our center's standard practice of reducing IS during episodes of CMV infection, while immunosuppression modifications were not clearly described in the SOLSTICE study.

Our study cohort is also larger than other real-world non-comparison studies that have reviewed outcomes with MBV [13,14,19]. These studies have all raised concerns about virologic failure during treatment with MBV and the development of MBV-specific resistance. In this study, although there was a trend towards higher rates of MBV resistance (18.5%), this was not a statistically significant difference when compared to rates of FOS resistance (11.1%). Another issue raised by the SOLSTICE study was that the study population primarily consisted of patients with low CMV viral loads, which has raised the question of the efficacy of MBV in those with high levels. We found no negative impact in terms of treatment success or failure by viral load in patients receiving MBV. However, it is possible that clinicians avoided MBV use in patients with higher viral load, given the concern that high viral load correlates with immune incompetence. Future directions would include additional immunologic testing to determine whether immune incompetence was a primary reason behind failure.

Ongoing comparison studies can help shed light on preferred agents for prophylaxis and treatment of asymptomatic disease, end-organ disease, or R/R CMV infection. A low ANC and/or ALC may reflect either CMV disease or a negative impact of GCV/VGC, which may in turn further negatively impact the immune control of CMV. Therefore, it is

possible that an earlier switch to a non-myelotoxic regimen would be beneficial, but this is an area that needs further study.

Our study was limited due to the small cohort size, heterogeneous patient population, and retrospective design. Although there were no significant baseline differences between groups, a larger sample size would have enabled a multivariable analysis to control for possible confounding factors, such as peak viral load, CMV end-organ disease, or asymptomatic DNAemia. We were also limited to the interpretation of clinical documentation regarding indications for choice of antivirals, reasons for switches in therapy, and use of oral versus intravenous formulations. In addition, our study population was based on individuals who had prescriptions at our institution and may have missed patients who received a prescription for MBV from an outside pharmacy. The optimal duration of antiviral treatment was individualized based on clinician discretion. Some patients were continued on secondary prophylaxis with MBV and subsequently developed breakthrough infection. More data is necessary to guide the optimal dosing and duration of treatment with MBV while limiting the risk of developing resistance mutations. Future studies are also needed to evaluate the impact of transplant type, high viral loads, and CMV end-organ disease on MBV treatment outcomes. However, these limitations are somewhat mitigated, as this was a single-center study with access to granular data regarding CMV infection, laboratory abnormalities, and response to therapy. Furthermore, the use of standardized internal protocols across time for all transplant recipients led to our ability to identify MBV and FOS cohorts that were well-matched in terms of demographic and clinical characteristics.

In summary, in a real-world cohort, MBV was used to successfully treat CMV infection with an increased success rate at our center compared with published data. Compared to FOS, MBV demonstrated no significant difference in the development of resistance and also had a trend towards a lower rate of recurrent infection within 8 weeks of completing therapy. MBV therefore presents an attractive alternative antiviral option for the treatment of R/R CMV infection and/or for patients who are unable to tolerate conventional therapy since it appears to be as effective and better tolerated than FOS.

Author Contributions: Conceptualization, L.O. and J.M.S.; methodology, L.O., C.M., C.P. and J.M.S.; validation, J.M.S.; formal analysis, J.M.S.; investigation, L.O. and C.M.; data curation L.O., C.M. and J.M.S.; writing—original draft preparation, L.O. and C.M.; writing—review and editing, L.O., C.M., A.M., O.E.B., P.G., C.P. and J.M.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: This study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board of UCLA (protocol code IRB-21-1043 approved on 1/9/24).

Informed Consent Statement: Not applicable.

Data Availability Statement: The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy and ethical restrictions and the local institution's data policy.

Conflicts of Interest: The authors declare no conflict of interest.

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Article

The Association Between Cytomegalovirus Infection and Kidney Damage in the Liver Transplant Setting

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Abstract: Introduction: The development of chronic kidney disease (CKD) is a common and significant complication, contributing to morbidity after liver transplantation (LT). Cytomegalovirus (CMV) infection is common in the overall population, and relevant reinfection after LT may occur. CMV-associated kidney damage has been discussed, but the clinical significance on CKD development after LT remains unclear. Methods: A total of 745 patients who underwent LT between 2006 and 2017 were included in this retrospective analysis. Clinical data, as well as laboratory parameters, were analyzed. Univariate and multivariate analysis were performed. Results: The univariate analysis revealed significantly impaired estimated glomerular filtration rates (eGFRs) in patients with histories of CMV infection (81.4 (8–137) mL/min vs. 90.0 (5–147) mL/min; $p = 0.004$). This effect was confirmed in the multivariate analysis. Post-LT, eGFR was impaired in patients with CMV (re)infection at 6, 12, 36, and 60 months, 10 years, and 15 years after LT. Immunosuppressive levels were comparable between groups. Overall survival was negatively affected by CMV infection ($p = 0.001$). Discussion: A clinically significant detrimental impact of CMV infection on renal function was observed, that could individualize clinical risk evaluation prior and after LT further. However, the pathophysiological mechanisms behind this observation are not yet understood.

Keywords: liver transplantation; cytomegalovirus; chronic kidney disease; immunosuppression

1. Introduction

Liver transplantation (LT) remains the only curative therapeutic option for various end-stage liver diseases (ESLDs) [1,2].

Post LT, lifelong immunosuppression (IS) remains standard therapy to prevent rejection episodes. IS itself is associated with short- and long-term complications such as chronic kidney disease (CKD), metabolic diseases, cardiovascular events, opportunistic infections, and malignancies [3–10].

Renal dysfunction and the diagnosis of CKD prior to LT have been established as independent risk factors for poorer outcomes after LT [11]. Via the hepatorenal pathway, kidney function can be impaired by the underlying liver disease and is therefore part of scoring systems, such as the Model for End-stage Liver Disease (MELD) [12,13]. Thus, the presence of CKD after LT is common and multifaceted, caused by liver disease itself and aggravated by, e.g., immunosuppression and decreasing renal function due to age [14,15]. Importantly, CKD has been linked to increased morbidity and decreased survival in patients [16–18].

CMV infection, as well as reactivation, after LT is one of the most relevant infections and it contributes to increases in morbidity and mortality [10,19]. CMV serostatus mismatch

of LT donors and recipients has been established as a risk factor for reoccurring infection, and recommendations on the administration of pharmaceutical prophylaxis are based upon these laboratory findings [20–22]. CMV infection in healthy, immunocompetent individuals commonly resolves with only minor symptoms, but critically ill, immunocompromised patients are at heightened risk for severe courses [23–25]. The range of manifestations of CMV infections is wide, with uncharacteristic symptoms such as fever in addition to colitis, encephalitis, pneumonitis, and nephritis with proteinuria [24,26].

Impairment of graft function in kidney transplant (KT) patients with CMV infection is generally recognized, but its mechanism is not fully understood and ranges from induction of rejection to endothelial alterations [27]. Interestingly, CMV infection has been linked to increase arterial stiffness in CKD patients [28]. The extent to which glomerulopathy is caused by CMV infection in non-KT patients remains disputed, and its clinical significance is unknown [29–33].

To our knowledge, the significance of CMV infection in the development of CKD in LT patients has not been thoroughly studied. This work aims to explore the potential link between CMV infection and renal dysfunction in LT patients and to contextualize these findings within long-term outcomes following LT.

2. Materials and Methods

All adult patients undergoing LT at a single transplant center between 2006 and 2017 were considered for this retrospective study. Follow-up for this study was ended in 12/2023. Patients with multiorgan transplantation, re-transplantation, and missing data were excluded.

Clinical and laboratory parameters, such as MELD score, CMV serostatus of donor and recipient, dosage and trough levels of IS, estimated glomerular filtration rate (eGFR) prior to LT, and clinical course after LT, were extracted from our prospectively maintained database. Life-long follow-up after LT was ensured via our outpatient clinic according to a standardized schedule ranging from twice a week to every three months, depending on time after LT. Renal function was assessed preoperatively within one month prior to LT and 6, 12, 36, 60, 120, and 180 months after LT. Classification of CKD was conducted using the Kidney Disease: Improving Global Outcomes (KDIGO) guidelines for eGFRs [34]. To further analyze the course of renal function, loss of eGFR was calculated by subtracting the means of the eGFRs.

To analyze comorbidities relevant to the development of CKD, the presence of "cardiovascular disease" was stated when patients were diagnosed with arterial hypertension on medication or atherosclerosis (e.g., coronary sclerosis, peripheral arterial disease). Similarly, "diabetes" was acknowledged if patients received antidiabetic drug treatment. Age at time of LT was dichotomized into $\leq$60 and >60 years.

CMV infection after LT was diagnosed by positive CMV-DNA-PCR, and was classified as follows: (i) CMV viremia in asymptomatic patients; (ii) CMV syndrome in patients with typical signs and symptoms of the disease; (iii) histologically proven tissue-invasive CMV infection.

CMV prophylaxis consisted of acyclovir or valganciclovir (VGCV) and was administered as part of standard of care according to donor/recipient CMV-IgG constellation up to 6 months after LT [35]. Routinely, preemptive therapy was conducted in new-onset viremia, but individual factors were acknowledged.

IS was administered in an individualized manner due to patients' risk profiles and comorbidities. Standard regimen consisted of calcineurine inhibitors (CNI; tacrolimus, cyclosporin A) with the addition of, e.g., mycophenolate mofetil (MMF), mammalian target of rapamycin inhibitors (mTORI), or glucocorticoids (GCs). GCs were routinely administered after transplantation and tapered until 12 weeks after LT. We focused on dosage and trough levels of tacrolimus and these values were analyzed within 2 weeks and 3, 6, 12, 36, 60, and 120 months after LT. Cumulative dosage was calculated by using the area under the curve (AUC). The impact of cumulative dosage on renal function was

calculated using the 50th percentile for each time point and, accordingly, patients were subsumed into a "low exposure" group and a "high exposure" group.

For descriptive analysis, absolute numbers (*n*) with percentages (%) were given with mean and standard deviation (SD) for normally distributed variables, or they were given as median with minimum and maximum for non-normally distributed variables. Cross tables were used in nominal variables and a *t*-test was used for normally distributed continuous variables. In cases of non-normally distributed values, a Mann–Whitney U-test or a Kruskal–Wallis test were performed. Pearson correlation was applied for metric variables. ANOVA was applied for the comparative analysis of multiple groups. For multivariate analysis, multinominal logistic regression was used to evaluate effect strength, and regression coefficient (β) and confidence interval (CI) were calculated. A two-sided *p*-value of <0.05 was considered significant. Statistical analysis was performed using SPSS Statistics Version 26.0 (IBM Co., Armonk, New York, NY, USA). Figures were created using SPSS and Microsoft Excel 2016. This study was approved by the ethics committee of Charité Universitätsmedizin, Berlin (protocol code EA1/255/20; date of approval: 20 October 2020).

3. Results

3.1. Cohort Characteristics

Overall, 745 patients undergoing LT between 2006 and 2017 were included in this study. The majority, 494 patients (66.3%), were male and the median age at transplantation was 56.3 (19.3–74.2) years. Median eGFR prior to LT was 84.80 (5–150) mL/min.

IS after LT was mainly CNI-based (*n* = 725/97.3%). At the end of follow-up, 313 (42.0%) patients were deceased and median survival after LT was 121.0 (6–213) months. For details on the patient cohort, see Table 1.

Table 1. Patient cohort's characteristics.

	Patient Cohort (*n* = 745)
Median age at LT in years (min–max)	56.3 (19.3–74.2)
Sex (%)	
Male	494 (66.3)
Female	251 (33.7)
Indication for LT (%)	
ALD	304 (40.8)
Viral hepatitis	168 (22.6)
AIH/PSC/PBC	97 (13.0)
NASH/cryptogenic cirrhosis	58 (7.8)
HCC/CCA	32 (4.3)
ALF	25 (3.4)
Others	61 (8.2)
Median MELD (min–max)	15 (6–40)
Comorbidities at LT (%)	
Cardiovascular disease	79 (10.6)
Diabetes	65 (8.7)
Median eGFR at LT mL/min (min–max)	84.8 (5–150)
CKD at LT (%)	
I	346 (46.4)
II	237 (31.8)
III	118 (15.8)
IV	28 (3.8)
V	16 (2.1)

Table 1. *Cont.*

	Patient Cohort (*n* = 745)
Donor-recipient CMV risk stratification (%)	
R negative/D negative	82 (11.0)
R negative/D positive	146 (19.6)
R positive/D negative	174 (23.4)
R positive/D positive	257 (34.5)
Missing data	86 (11.5)
CMV infections after LT (%)	271 (36.4)
Time from LT	
<90 days	220 (81.2)
>90 days	51 (18.8)
Median viral load in IU/mL (min–max)	
At diagnosis	3160 (10–13,900,000)
Peak	7335 (756–13,900,000)
Manifestation (%)	
CMV viremia	203 (27.2)
CMV syndrome	66 (8.9)
CMV tissue invasion	2 (0.003)
Treatment (%)	
Observation only	61 (22.5)
IS reduction	34 (12.5)
(val)ganciclovir	176 (64.9)
Maintenance IS after LT (%)	
CNI	725 (97.3)
Tacrolimus	625 (83.8)
Cyclosporin A	100 (13.4)
MMF	353 (47.4)
mTORI	81 (10.9)
Combination therapy	476 (63.9)
Mean time of follow-up in months (SD)	117.2 (58.3)
Patients deceased (%)	313 (42.0)
Cause of death	
Graft failure	38 (5.1)
Neoplasms	108 (14.5)
Cardiovascular	36 (4.8)
Pulmonary	9 (1.2)
Infection	68 (9.2)
Neurological	7 (0.9)
Acute bleeding	10 (1.3)
Trauma	5 (0.7)
Other	32 (4.3)

LT—liver transplantation; ALD—alcoholic liver disease; AIH—autoimmune hepatitis; PSC—primary sclerosing cholangitis; PBC—primary biliary cirrhosis; NASH—non-alcoholic steatohepatitis; HCC—hepatocellular carcinoma; CCA—cholangiocellular carcinoma; ALF—acute liver failure; eGFR—estimated glomerular filtration rate; CKD—chronic kidney disease; CMV—cytomegalovirus; CNI—calcineurin inhibitor; MMF—mycophenolate mofetil; mTORI—mammalian target of rapamycin inhibitor.

3.2. CMV Infection and Renal Function Prior to LT

Patients with positive CMV-IgG serostatus showed significantly lower median eGFR than patients without prior CMV infection ($p = 0.004$). Patients aged $\leq$60 had higher median eGFR compared to patients > 60 years ($p = 0.011$). Female patients showed significantly lower median eGFR ($p < 0.001$). Presence of cardiovascular disease at LT was also significantly associated with lower median eGFR ($p < 0.001$). Furthermore, the underlying disease leading to LT was associated with significant differences in median eGFR. MELD score showed statistically significant inverse correlation with eGFR ($r = -0.323$, $p < 0.001$). For details of variables, see Table 2.

Table 2. Impact of clinical parameters on eGFR and CKD prior to LT in univariate analysis. Clinical variables with putative impact on renal function using either the CKD classification or eGFR were explored.

Variable	Groups	CKD I *n* (%)	CKD II *n* (%)	CKD III *n* (%)	CKD IV *n* (%)	CKD V *n* (%)	*p*	eGFR Prior to LT in mL/min Median (Min–Max) *	*p*
Age	≤60 years	254 (50.3)	147 (29.1)	72 (14.3)	21 (4.2)	11 (2.2)	0.019	90.0 (8–150)	$p = 0.011$
	>60 years	92 (38.0)	90 (38.0)	46 (19.2)	7 (2.9)	5 (2.1)		81.4 (11–131)	
Sex	Male	256 (51.8)	155 (31.4)	67 (13.6)	13 (2.6)	3 (0.6)	<0.001	90.0 (10–150)	<0.001
	Female	90 (35.9)	82 (32.7)	51 (20.3)	15 (6.0)	13 (5.2)		71.4 (5–137)	
Indication for LT	ALD	128 (42.1)	106 (34.9)	57 (18.8)	9 (3.0)	4 (1.3)	0.005	82.7 (10–123)	0.038
	Viral hepatitis	74 (44.0)	60 (35.7)	25 (14.9)	6 (3.6)	3 (1.8)		83.0 (12–131)	
	AIH/PSC/PBC	55 (56.7)	29 (29.9)	9 (9.3)	1 (1.0)	3 (5.2)		90.0 (11–133)	
	NASH/cryptogenic cirrhosis	24 (41.1)	16 (27.6)	12 (20.7)	3 (4.9)	3 (5.2)		69.5 (8–137)	
	HCC/CCA	20 (62.5)	8 (25.0)	2 (6.3)	1 (3.1)	1 (3.1)		90.5 (12–135)	
	ALF	13 (52.0)	4 (16.0)	3 (12.0)	5 (20.0)	0 (0)		90.0 (17–150)	
	Others	32 (52.5)	14 (23.0)	10 (16.4)	3 (4.9)	2(3.3)		90.0 (5–147)	
Cardiovascular disease	-	23 (29.1)	30 (38.0)	20 (25.3)	5 (6.3)	1 (1.3)	0.008	62.0 (11–118)	<0.001
								88.1 (5–150)	
Diabetes	-	29 (44.6)	23 (35.4)	13 (20.0)	0 (0.0)	0 (0.0)	0.256	78.4 (30–130)	0.82
								85.4 (5–150)	
Recipient CMV status	CMV positive	209 (43.8)	153 (32.1)	82 (17.2)	24 (5.0)	9 (2.4)	0.059	81.4 (8–137)	0.004
	CMV negative	132 (52.0)	77 (30.3)	35 (13.8)	4 (1.6)	6 (1.9)		90.0 (5–147)	
Median MELD	-	14 (6–40)	14 (6–40)	20 (6–40)	32 (15–40)	36 (20–40)	<0.001	r = −0.323 **	<0.001

LT—liver transplantation; ALD—alcoholic liver disease; AIH—autoimmune hepatitis; PSC—primary sclerosing cholangitis; PBC—primary biliary cirrhosis; NASH—non-alcoholic steatohepatitis; HCC—hepatocellular carcinoma; CCA—cholangiocellular carcinoma; ALF—acute liver failure; CMV—cytomegalovirus; MELD—model of end-stage liver disease. * median and range only for categorical variables. ** Pearson correlation coefficient was calculated for correlation of metric MELD and eGFR).

Analysis of influence on kidney function prior to LT according to CKD stage showed similar results. However, distribution of renal function according to CKD classification did not significantly differ with regard to prior CMV infection ($p = 0.059$); for details, see Table 2.

Multivariate analysis confirmed significantly higher eGFR in patients < 60 years ($p < 0.001$) at LT and male patients ($p < 0.001$). EGFR was significantly lower in patients diagnosed with cardiovascular disease ($p = 0.004$) and higher MELD score ($p < 0.001$) in this analysis. CMV infection prior to LT did not show any statistically significant association with eGFR levels (see Table 3).

Table 3. Multivariate analysis of impact of variables on eGRF prior to LT.

Variable	*p*	Regression Coefficient	95% CI	
			Lower	Upper
Age (ref.: ≤60 years)	<0.001	−7.29	−11.42	−3.17
Sex (ref.: male)	<0.001	−12.52	−16.70	−8.34
Indication for LT	0.015	1.49	0.29	2.70
Cardiovascular disease (ref.: yes)	0.004	8.90	2.84	14.96
Recipient CMV status (ref.: CMV negative)	0.117	−3.22	−7.25	0.81
MELD	<0.001	−0.88	−1.07	−0.69

LT—liver transplantation; CMV—cytomegalovirus.

3.3. CMV Infection and Renal Function Post LT

EGFR after LT showed a slight increase within 6 months from a median of 84.80 (5–150) mL/min prior to LT to 86.2 (8–139) mL/min ($p > 0.001$) and a continuous decrease in the following years to 61.0 (5–118) mL/min after ten years. Statistically significant inverse correlation of MELD score and eGFR was found 6 and 12 months after LT ($p = 0.008$ and $p = 0.006$, respectively) but not at a later timepoint. Using the CKD classification, significantly higher MELD scores were observed in patients with declining kidney function 6 and 12 months ($p = 0.002$ and $p = 0.002$) after LT but not at a later point in time.

Statistically significant decreased eGFR was observed in patients with post-LT CMV infection 6 months ($p = 0.002$), 12 months ($p = 0.024$), 36 months ($p = 0.003$), 60 months ($p = 0.001$), 10 years ($p = 0.037$), and 15 years ($p = 0.032$) after LT. Of note, comparison of these groups showed significantly higher eGFR prior to LT in patients without post-LT CMV infections (87.5 (5–150) vs. 79.9 (10–137) mL/min, $p = 0.007$). Total difference in median eGFR was 3.75 mL/min 6 months after LT and increased to 6.9 mL/min after 5 years and to 6 and 32 mL/min after 10 and 15 years, respectively.

Using the CKD classification, similar results were found: patients with post-LT CMV infection showed significantly higher stages at 6 months (CKD stage > 2: 80 (17.3%) vs. 68 (26.0%), $p = 0.006$), 3 years (CKD stage > 2: 75 (19.6%) vs. 60 (29.3%), $p = 0.05$), and 5 years (CKD stage > 2: 72 (22.6%) vs. 61 (36.5%), $p = 0.008$) after transplantation.

No difference between these two groups were found regarding age, sex, cardiovascular disease, or diabetes. Indication leading to LT showed fewer alcoholic ESLD (41.4% vs. 39.9%) and viral hepatitis (25.9% vs. 16.6%) patients developing post-LT CMV infection than patients with autoimmune diseases (12.2% vs. 14.4%) and cryptogenic cirrhosis (5.9% vs. 11.1%) ($p = 0.003$). Patients who developed a post-LT CMV infection had a higher median MELD score (14 (6–44)) vs. 18 (6–48), $p < 0.001$).

No difference was found in regard to the extent of infection (viremia vs. syndrome vs. tissue invasion) and kidney function at any time point, neither through analyzing eGFR nor CKD stages. The extent of viremia was not associated with changes in eGFR or CKD stage at any time point. A subgroup analysis of 47 patients with more than one episode of new viremia after LT vs. 224 patients with only one episode of post-LT CMV infection showed that statistically lower eGFRs were only present ten years after transplant. Here, multiple recurrent infections were associated with lower eGFR (58.0 (16–118) vs. 40.0 (8–95) mL/min, $p = 0.018$). All patients with more than one episode of post-LT CMV infection were deceased 15 years after LT.

For further analysis, three groups were defined: group 1—patients with CMV-negative serostatus at the time of LT and no recorded post-LT CMV infection; group 2—patients with seropositive CMV status at the time of LT without post-LT CMV infection; group 3—all patients with post-LT CMV infection regardless of the preoperative serostatus (see Figure 1).

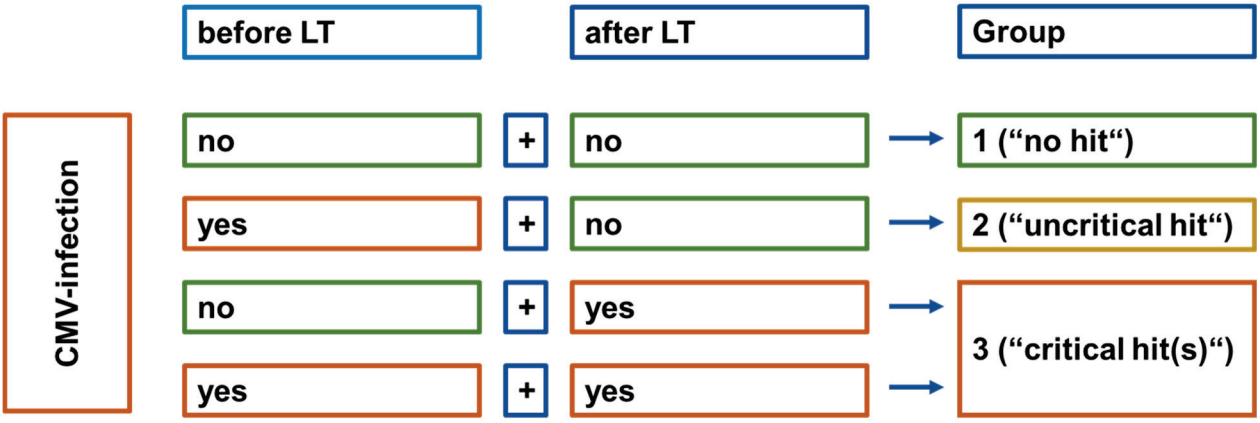

Figure 1. Constitution of groups for analysis of impact of CMV infection. For the analysis of subgroups in regard to exposition to CMV, three groups were defined. Group 1 is formed by patients without any recorded CMV infection; in group 2, patients were only CMV-IgG-positive at LT; group 3 was formed by patients that had recorded CMV infection after LT.

No difference was observed between groups regarding age, sex, or comorbidities, but MELD score was different with 13 (6–40) in group 1, 15 (6–40) in group 2, and 18 (6–40) in group 3 ($p < 0.001$).

eGFR between these groups differed prior to LT with statistical significance, as group 1 showed better median eGFR with 90.0 (5–150) mL/min than group 2, with a median eGFR of 84.3 (8–137) mL/min, and group 3 with 79.9 (10–137) mL/min ($p = 0.005$). This observation remained statistically significant throughout the follow-up of 6 months, 3 years, and 5 years ($p = 0.005$, $p = 0.011$, and $p = 0.005$, respectively). In the long-term follow-up, however, 10 and 15 years after LT, this effect did not reach statistical significance anymore ($p = 0.11$ and $p = 0.11$, respectively).

Analyzing renal function using the CKD classification showed no statistically significant differences in the distribution of the CKD stages between the groups at the time of transplant ($p = 0.071$). Six months after LT, CKD stages ≥ 3 were found more frequently in group 3 (25.9%) vs. group 2 (17.5%) or group 1 (17.0%) ($p = 0.015$). This statistically significant difference in CKD stages was not found at the timepoints of 1 and 3 years, but was found again at 5 years ($p = 0.038$) after LT. For details on the course of eGFR, see Figure 2 and Supplementary Materials, Table S1.

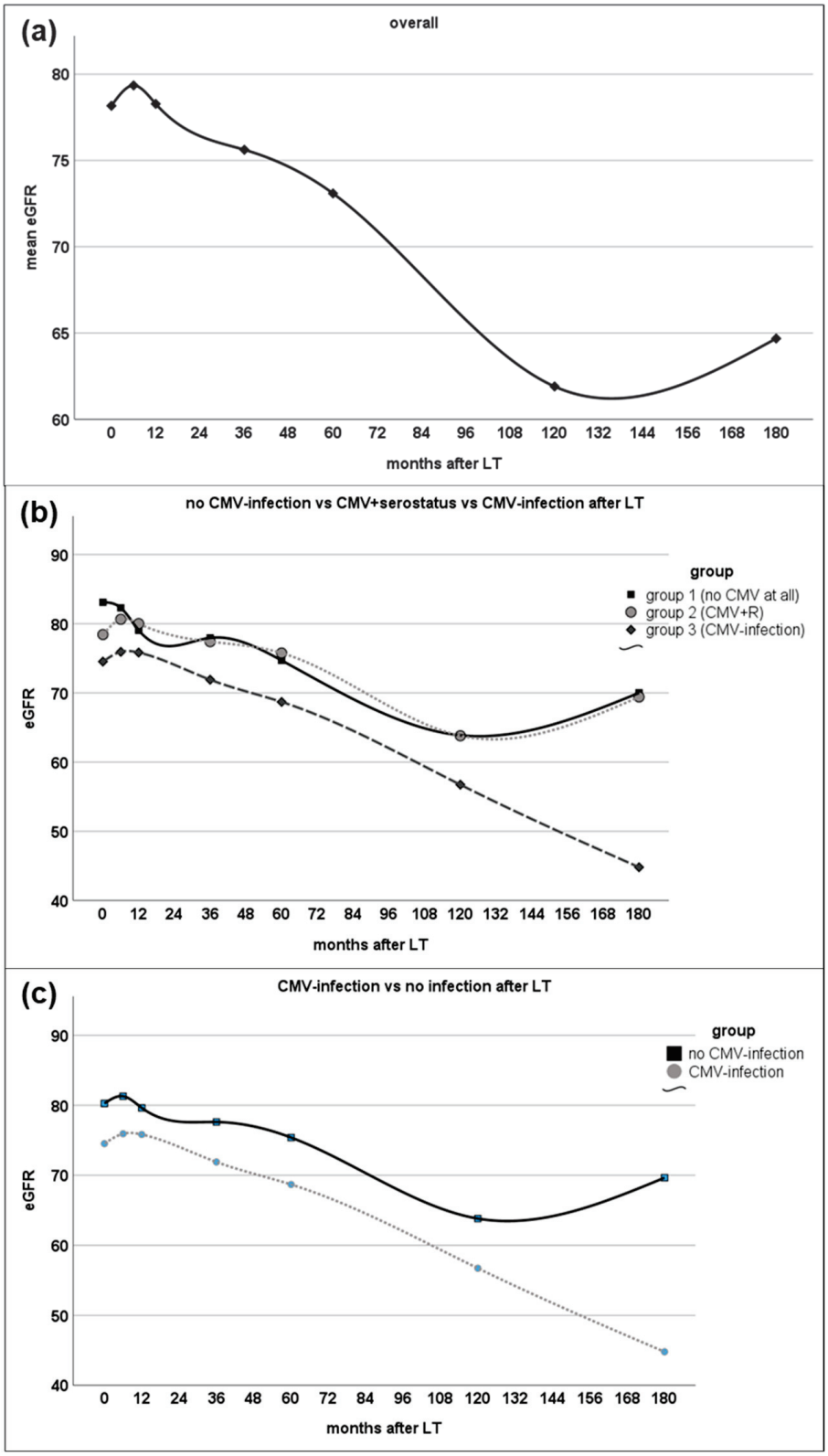

Figure 2. Course of median eGFR over time. There was a decrease in overall renal function regarding median eGFR over time in the overall cohort (**a**). eGFR showed different dynamics depending on occurrence of CMV infection (**b**,**c**). For details on eGFR at given time points, see Supplementary Materials, Table S1. LT—liver transplantation; CMV—cytomegalovirus; ~ indicates interpolation line.

3.4. Immunosuppression and Renal Function

To assess the impact of immunosuppression on kidney damage, the dosage of CNI was compared between groups. Mean tacrolimus trough level as well as cumulative dosage showed no difference between patients with or without CMV infection after LT at any time point. Cross-testing of dichotomized cumulative dosage was conducted, using the 50th percentile as the cut-off; here, statistically significantly lower dosages were found more frequently in the patients with CMV infection at 6 months after LT ($p = 0.009$) but not beyond.

Comparison of patients with no CMV infection (group 1), patients with CMV seropositivity only (group 2), and those with CMV infection (group 3) also showed no significant difference in mean tacrolimus trough level or cumulative exposure to CNI. Here, dichotomized tacrolimus exposure again revealed the lowest cumulative tacrolimus dosage in group 3 with statistical significance only at 6 months and 12 months after LT ($p = 0.015$, $p = 0.014$).

Analysis of "low" or "high" tacrolimus exposure and CKD classification showed significant differences between these groups only 12 months after LT: here, the group of "low exposure" consisted of more patients with impaired renal function (CKD > 1, $p = 0.011$). No difference in eGFR was found at any time point after LT between patients undergoing rejection prophylaxis with monotherapy or combination therapy. Analysis of IS regimen (monotherapy vs. combination therapy) showed no significant differences on CMV occurrence after LT. Usage of mTORI in IS regimen revealed a trend towards decreased CMV infections ($n = 25$ (28.7%) vs. $n = 246$ (37.6%), $p = 0.067$).

3.5. CMV Infection and Overall Survival

Median survival after LT was significantly longer in seropositive CMV (CMV + R) patients prior to LT compared to seronegative recipients (128.0 (6–213) vs. 110.5 (6–113) months, $p = 0.048$). However, CMV infection after LT was associated with an impaired overall survival of 114.0 (0–210) compared to 126.5 (6–213) months ($p = 0.001$). This observation was confirmed using a Kaplan–Meier analysis (Log rank 0.047). A similar difference was found between the three groups. Patients with a history of CMV infection prior to LT (group 2) had the highest median survival of 129.0 (6–213) months, followed by patients without any CMV infection at all with 117.0 (7–213) months. Patients with CMV infection after LT (group 3) showed shortest median survival of 114.0 (6–210) months ($p = 0.003$). In Kaplan–Meier analysis, this observation did not reach statistical significance ($p = 0.12$). Patients with more than one CMV infection after LT also showed decreased median survival (81.0 (6–177) vs. 120.5 (6–210) months, $p = 0.001$), and this effect was confirmed in the Kaplan–Meier analysis (log-rank < 0.001) (see also, Figure 3). Malignancies were the most common cause of death in groups 1 (58.2%) and 2 (45.9%), whereas infections were the most common cause of death in group 3 (34.3%, $p = 0.029$). MELD score did not correlate with overall survival ($p = 0.815$).

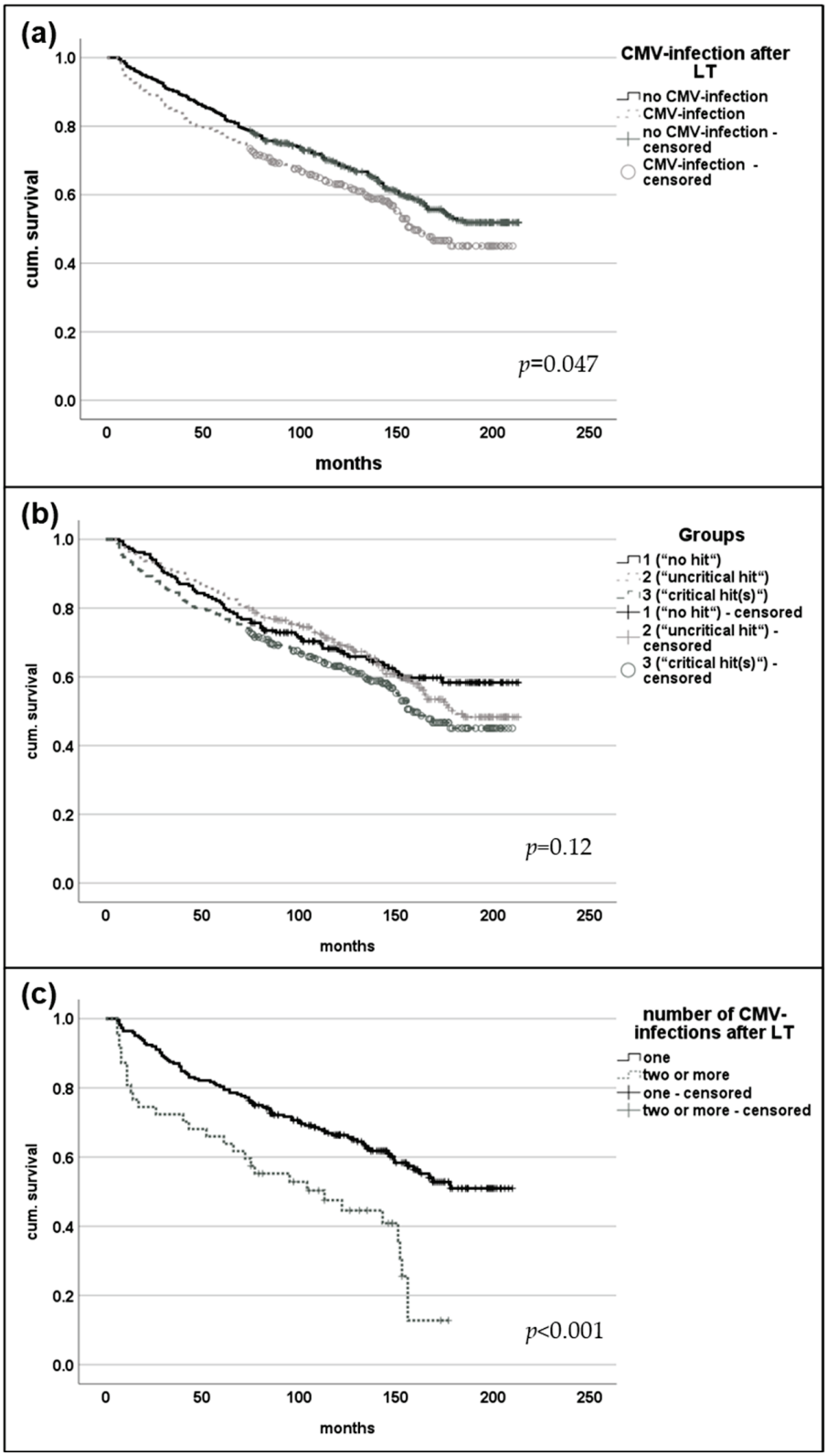

Figure 3. Survival analysis after LT. Kaplan–Meier analysis revealed significantly impaired overall survival after LT in patients with recorded CMV infection after transplantation (**a**). Subgroup analysis for patients without any recorded CMV exposure (Group 1) compared to patients with CMV IgG positivity prior to LT (group 2) or patients with CMV infection after LT (group 3) did not show statistically significant differences (**b**). Patients with more than one episode of CMV viremia were found to have impaired overall survival after LT (**c**).

4. Discussion

In this study, reporting on 745 LT patients from a single center, a significant impact of CMV infection after LT and reduced kidney function was found up to 15 years after transplantation.

Reduced glomerular filtration rate was observed in patients with a history of CMV infection prior to LT, as defined by IgG positivity in univariate but not multivariate analysis. Other potentially confounding factors (age, sex, indication for LT) also showed significant association to reduced kidney function, but diagnosis of diabetes at the time of transplantation did not. However, we did not assess the severity of diabetes (e.g., insulin dependency) or new-onset diabetes during follow-up. Of note, we found a strong correlation of MELD score and renal function up to 12 months after LT but not on overall survival. Correspondingly, higher MELD scores have been associated with more frequent acute kidney injury and even increased short-term mortality after LT [36–38].

In this study, CMV-IgG-positive patients showed lower eGFR prior to LT compared to those without. Prevalence of CMV worldwide is high, ranging between 50 and 100%, and infection is usually successfully controlled by the immune system in the immunocompetent population [39,40]. Of note, CMV serology cannot predict immunity [41]. Furthermore, reduced renal function in patients with ESLD is common and associated with impaired outcome [11]. While only few reports of CMV-related kidney damage in immunocompetent patients exist, a link to glomerulopathy and arterial stiffness has been made [28,29,31]. Also, CMV copies in the urine sediment in non-immunocompromised patients with acute kidney injury have been reported [42]. While renal damage in pre-LT patients is certainly multifaceted, it is noteworthy that ESLD patients can be classified as immunocompromised with dysfunction in both innate and acquired immunity [43–45]. CMV seropositive cirrhotic patients have been reported to have an elevated mortality [46]. The impact of CMV infection before and after LT on the development of CKD in LT patients is not very well known. In this study, significantly reduced eGFR was found in patients with CMV infection after LT and in the group of patients with recurrent CMV exposition compared to CMV-negative patients. However, these groups showed differences in renal function even prior to LT. This might be due to the fact that there is a well-known significant overlap of CMV seropositive patients prior to LT and infection after LT, as CMV seropositivity contributes to a higher rate of reinfection after transplantation [47–49]. International recommendations for prophylactic treatment regimens take these risk constellations into account [20,48,50,51]. No association between amounts of episodes of CMV viremia after LT or extent of CMV infection and kidney damage was observed in this cohort. Given the relatively low numbers of patients in the group of CMV syndrome ($n = 66$) and tissue invasion ($n = 2$), the effect of extent of CMV infection might have been missed in statistical analysis. Additionally, the CMV-induced renal damage might not be connected to the typical clinical presentation of the infection.

The mechanism and dynamics of the hypothesized CMV-induced damage on glomeruli remain unclear and were not investigated in this work. However, results from this study and clinical data would rather suggest a "slow-burning" processes linked to pathophysiology with subclinical manifestation rather than fulminant kidney injury. This is supported by findings of persistent elevated mortality in patients with CMV infection after liver or kidney transplantation in the long-term setting, despite the early occurrence of infection post-transplantation [52–54]. Direct glomerulopathy as well as, e.g., affection of vessels, has been proposed to contribute to kidney damage in CMV infection [55–58]. Others have evaluated CMV-induced glomerulopathy rather as episodes or manifestation of rejection that have been misidentified in the context of KT patients [59]. As mentioned above, in LT patients, negative effects of CMV infection are widely known, but the affection of the kidney is not the focus [47,60]. Assessing different "groups at risk" showed clinically relevant impaired kidney function in patients with histories of CMV infection prior to LT and CMV infection after LT, even when compared to those with a history of CMV infection prior to LT only. This might suggest additional, cumulative damage caused by CMV infection, as relevant confounders such as age and sex were ruled out. Certainly, multiple factors

contribute to renal damage in an additive maybe even exponential character and especially in multimorbid patients with ESLD or after LT. Weighting of factors is difficult, but CMV infection might mean an additional "hit" in a multifactorial process.

CNIs, especially tacrolimus, were the backbone IS in this cohort and are known for their dosage-dependent nephrotoxicity [61]. However, this potentially major confounder was not present in this study, as mean levels and cumulative dosages were not higher in patients with impaired renal function. On the contrary, significantly lower CNI exposure was observed 6 months after LT, indicating proper trough-level-dependent monitoring and individualized adjustment of IS.

In summary, these findings highlight the importance of prevention of CMV reactivation in the post-transplant setting and underline the effect of individualized regimens in terms of prevention strategies, closely linked to a well-observed individualized immunosuppressive therapy.

Certain limitations must be mentioned. Although the cohorts mostly represent current standards of diagnostics and treatment, its retrospective nature might have inherited relevant additional confounders in diagnostics and therapeutical concepts that were not exposed and therefore not taken into account in statistical analysis. Renal function prior to LT is certainly affected by various aspects including comorbidities and, e.g., socioeconomic aspects, that were not all assessed in this study. Also, the fundamental question of the pathophysiological mechanisms of CMV-induced renal damage was not explored in this study; thus, at this point, only its clinical observation remains. However, as it seems to be of relevance, current routine laboratory testing is relevant in day-to-day care; patients with new CMV infection and even patients who have undergone CMV infection might profit from further individualized risk stratification.

5. Conclusions

CMV infection before and after LT might contribute to the pathogenesis of CKD in the LT population and therefore play a pivotal role in contributing to major comorbidities that are relevant in the setting of aftercare. However, the pathophysiological mechanisms remain unclear; their identification requires further clinical and experimental research.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/v16121830/s1, Table S1: Course of eGFR after LT, dependent on CMV infection.

Author Contributions: Conceptualization, D.E., E.M.D., L.D. and R.R.O.S.; methodology, R.R.O.S., F.E. and L.D.; validation, B.G., R.Ö. and W.S.; formal analysis, R.R.O.S. and L.D.; investigation, F.E., L.D. and R.R.O.S.; resources, J.P.; data curation, F.E., R.R.O.S., N.R. and D.E.; writing—original draft preparation, L.D., R.R.O.S., E.M.D. and D.E.; writing—review and editing, B.G., N.R., W.S., R.Ö. and J.P.; visualization, R.R.O.S. and L.D.; supervision, J.P., E.M.D., D.E. and N.R.; project administration, D.E. and J.P. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: This study was approved by the ethics committee of Charité Universitätsmedizin Berlin (protocol code EA1/255/20; date of approval: 20 October 2020).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy and ethical restrictions and the local institution's data policy.

Conflicts of Interest: The authors declare no conflicts of interest.

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viruses

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Article

Association between Respiratory Virus Infection and Development of *De Novo* Donor-Specific Antibody in Lung Transplant Recipients

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Abstract: Chronic lung allograft dysfunction (CLAD) is the most common cause of long-term lung allograft failure. Several factors, including respiratory virus infection (RVI), have been associated with CLAD development, but the underlying mechanisms of these associations are not well understood. We hypothesize that RVI in lung transplant recipients elicits the development of donor-specific antibodies (DSAs), thus providing a mechanistic link between RVI and CLAD development. To test this hypothesis, we retrospectively evaluated for the presence of HLA antibodies in a cohort of lung transplant recipients with symptomatic RVI within the first four months post-transplant using sera at two time points (at/directly after the transplant and following RVI) and time-matched controls without RVI (post-transplant). We found a trend toward the development of *de novo* DSAs in those with symptomatic RVI versus controls [6/21 (29%) vs. 1/21 (5%), respectively, $p = 0.09$]. No cases or controls had DSA at baseline. We also found increased rates of CLAD and death among those who developed class II DSA versus those who did not (CLAD: 5/7 (71.4%) vs. 19/34 (54.3%), death: 5/7 (71.4%) vs. 17/35 (48.6%)). Prospective studies evaluating the temporal development of DSA after RVI in lung transplant patients and the subsequent outcomes are warranted.

Keywords: lung transplant; respiratory viral infection; HLA antibody; chronic lung allograft dysfunction; donor-specific antibody

1. Introduction

While advances in surgical techniques, immunosuppression and pre-transplant HLA matching have improved one-year survival following lung transplant, long-term outcomes remain poor. The most common cause of lung graft failure and death after the first-year post-transplant is chronic lung allograft dysfunction (CLAD), which develops in approximately half of all lung transplant recipients (LTRs) by five years post-transplant [1]. The pathogenesis of CLAD is poorly understood, with multiple potential triggers, including T cell-mediated acute cellular rejection, chemical insult (e.g., reflux), and certain infections. Recent studies have also suggested that *de novo* HLA donor-specific antibodies (DSAs) may predict CLAD development [2]. HLA class II DQ, in particular, has been associated with the obstructive form of CLAD (bronchiolitis obliterans syndrome, BOS) [3]. Animal models of organ transplantation suggest that *de novo* DSAs may develop via pathologic activation of the innate and adaptive immune system after allograft injury [4]. Respiratory virus infections (RVIs) are common in LTRs, can cause direct acute allograft damage, and have also been associated with the development of CLAD in multiple studies [5–17]. However, these previous studies have been more correlative and did not address the

potential mechanism(s) by which RVI may lead to CLAD, including the role of *de novo* DSAs in mediating chronic allograft damage after lung transplantation.

We hypothesize that RVI is linked to the development of pathologic *de novo* DSAs after lung transplantation, thereby providing a potential mechanism for the observed association of RVI with CLAD. To test this hypothesis, we retrospectively evaluated post-transplant *de novo* HLA antibodies from a biobank of longitudinal sera in a cohort of lung transplant recipients with either symptomatic RVI or matched controls without RVI and followed these patients long-term for development of CLAD and/or death.

2. Methods

2.1. Study Cohort and Design

We retrospectively identified 21 adult LTRs transplanted at the University of Washington between January 2007 and May 2012 who developed symptomatic RVI within the first 110 days post-transplant and who had serum available within 90 days prior to RVI (or at time of transplant) and within 6 months after RVI (cases). Cases were matched 1:1 to LTRs without symptomatic RVI during the same transplant time period (controls) based on the time of serum samples available post-transplant. Cases and controls were selected from a larger cohort of 250 LTRs, as outlined in the consort diagram (Supplementary Figure S1). To minimize bias, selection of cases and controls was based on the RVI status and sample availability only, and was performed blinded to clinical knowledge of patients, including pre-transplant HLA matching, DSA, and CLAD endpoints. Baseline demographic and transplant information were collected via electronic medical record review by trained personnel using standardized data collection forms. Some clinical information on the included patients has been previously published in a larger cohort study [5]; however, the prior study only examined the association between RVI and CLAD and did not examine DSA development. Furthermore, the current study is distinguished from prior work because it uses the updated 2019 consensus definitions for CLAD [18]. This study was approved by the University of Washington Institutional Review Board (IRB#44580).

2.2. Post-Transplant Follow-Up of LTRs

At the University of Washington, LTRs are followed closely for at least the first-year post-transplant, with outpatient visits occurring weekly for 4 weeks, every 2 weeks for 1 month, and every 2–3 months until 12 months post-transplant. Patients are instructed to perform home spirometry via a hand-held spirometer given to the patients at the time of their transplant, and they are instructed to tell their transplant team if there is a $\geq$10% decrease in the forced expiratory volume in one second (FEV1). Formal pulmonary function tests are performed with routine clinic visits and as clinically indicated. A decrease of $\geq$10% in FEV1 typically prompts investigation for underlying causes of this decline, including consideration for RVI testing if the patient has compatible symptoms. LTRs also typically have blood draws at least once a week for the first three months (i.e., for immunosuppression levels, CMV, PCRs, etc.); the sera used for this research study were obtained as part of a leftover sample biorepository. During the study period, approximately 262 lung transplants were performed at the University of Washington.

2.3. Respiratory Virus Testing

RVI testing was conducted only in patients who presented with upper and/or lower respiratory tract symptoms (e.g., fever, cough, rhinorrhea, coryza, sinus pain/pressure, sore throat, shortness of breath, etc.), had radiographic abnormalities, and/or had decreases in their spirometry. No surveillance testing in asymptomatic patients was performed. Since the decision to test for RVI was based on the clinician's discretion and was not performed specifically for a research study, no standardized symptom surveys were used. Nasal swabs, washes, or BAL specimens were tested for respiratory viruses using either a direct fluorescent antibody and culture or a laboratory-developed PCR assay that tests for 12 viruses: respiratory syncytial virus (RSV), parainfluenza (PIV) 1–4, influenza A and B,

adenovirus (ADV), coronavirus (CoV), rhinovirus (RHV), metapneumovirus (MPV), and bocavirus, as previously described [19–24]. Based on center protocols, BAL was performed with RVI testing if there was any concern for lower respiratory tract disease (i.e., abnormal chest imaging, more severe lower respiratory symptoms, such as shortness of breath, productive cough, or decrease in spirometry values). Given this testing algorithm, the RVI was considered lower tract if the respiratory virus was detected via BAL sample and upper tract if detected only by nasal swab or nasal wash.

2.4. Determination of CLAD

All available clinical, radiographic, and spirometry data were reviewed separately by two transplant pulmonologists who were blinded to the study results. In the case of disagreement in the diagnosis, the two reviewers conferred, and a consensus was reached in all instances. Per the 2019 International Society for Heart and Lung Transplant (ISHLT) Consensus Report, CLAD was defined as a decline in FEV1 to $\leq$80% of the patient's baseline value for >3 months in the absence of clinical confounders [25]. The CLAD phenotype at CLAD onset was determined based on the available clinical data up to three months after CLAD diagnosis. Per the consensus statement, four types of CLAD phenotypes were considered: bronchiolitis obliterans syndrome [BOS], restrictive allograft syndrome [RAS], mixed, and undefined. The definitions and diagnostic criteria for these phenotypes are discussed in depth in the ISHLT Consensus Report [25]. Only the phenotype present at the diagnosis of CLAD was used as an endpoint in this study; evolution of CLAD phenotypes (e.g., from BOS to RAS) at later time points was not examined.

2.5. Laboratory Testing and Determination of DSA

Sera from cases and controls were evaluated for anti-HLA DSA on FlowPRA beads representing the HLA-A, -B, -Cw, -DR, -DQ, and -DP antigens. HLA testing on the research sera was performed using OneLambda kits (Thermo Fisher Scientific, Waltham, MA, USA). DSA that was not previously present and crossed the mean fluorescence intensity (MFI) threshold of >1500 was termed new or *de novo* DSA, as per standard guidelines [26]. DSA testing was conducted by personnel blinded to clinical status (case vs. control, CLAD vs. no CLAD).

2.6. Statistical Analysis

The primary endpoint was the percentage of LTRs among cases versus controls, who developed *de novo* DSA between the baseline and the follow-up time points. The exploratory endpoint was the association between the development of *de novo* DSA and a composite endpoint of CLAD and death. The chi-squared test or Fisher's exact test (dichotomous) and Wilcoxon rank sum test (continuous) were used as applicable for the primary and exploratory endpoints and to compare baseline variables. Kaplan–Meier curves were also used to estimate and graph the probability of the composite endpoint of CLAD (including all sub-types) and death. Stata version 16 was used for all statistical analyses (StataCorp, College Station, TX, USA).

3. Results

3.1. Patient Cohort

Twenty-one LTRs with symptomatic RVI within the first 110 days post-transplant who also had sera available prior to and after the RVI episode were identified as cases, and twenty-one LTRs without symptomatic RVI and with similarly timed sera available were matched as controls (Supplementary Figure S1). Baseline demographic and transplant characteristics of the cases and controls are shown in Table 1; no statistically significant differences were seen. All patients underwent induction with Basiliximab per our center's protocol. Maintenance immunosuppression included the use of a calcineurin inhibitor (predominantly tacrolimus), an antimetabolite (mycophenolic acid or mycophenolate mofetil), and prednisone. The major indications for transplant in this population were chronic ob-

structive pulmonary disease, interstitial pulmonary fibrosis, and cystic fibrosis. The median (interquartile range, IQR) among the transplant, the baseline, and the follow-up serum samples are shown in Table 1. Cases and controls were matched based on the number of days post-transplant when the serum samples were available for HLA testing, and the median (IQR) difference between the case and matched control samples was 1 day (IQR: 1–3 days) and 6 days (IQR: 2–8) for the baseline and follow-up serum samples, respectively. In cases, the median time from transplant to RVI was 71 (IQR: 50–88) days, the median time from the baseline sample was 54 (IQR: 15–69) days prior to RVI, and the median time from RVI to the follow-up serum sample was 131 (IQR: 90–153) days. The median (IQR) follow-up time from transplant to death or last follow-up was 3268 (IQR 2372–3917) days, with no statistically significant difference between cases and controls. Additionally, all cases and controls had full follow-up for CLAD or death through at least 1000 days post-transplant.

Table 1. Baseline and sample characteristics in lung transplant recipients with and without RVI.

Characteristics	Cases, n = 21	Controls, n = 21
Age in years, median (IQR)	56 (51–64)	58 (45–63)
Female sex, n (%)	8 (38.1)	8 (38.1)
Underlying pulmonary disease, n (%)		
COPD/Bronchiectasis	8 (38.1)	6 (28.6)
IPF	6 (28.6)	6 (28.6)
Cystic Fibrosis	2 (9.5)	5 (23.8)
Other	5 (23.8) [a]	4 (19.0) [b]
Single lung transplant, n (%)	4 (19.0)	2 (9.5)
Year of transplant		
2007–2009	14 (66.7)	13 (61.9)
2010–2011	7 (33.3)	8 (38.1)
Days, transplant to first serum sample, median (IQR)	19 (15–23)	17 (13–21)
Days, transplant to second serum sample, median (IQR)	179 (154–225)	180 (154–225)

COPD: chronic obstructive pulmonary disease; IPF: idiopathic pulmonary fibrosis; [a] Alpha-1 antitrypsin (1), sarcoidosis (1), interstitial lung disease (3); [b] Alpha-1-antitrypsin (1), LAM (1), pulmonary hypertension (1), interstitial lung disease (1).

3.2. Details of Respiratory Virus Infection

The most common respiratory viruses in the RVI case cohort were seasonal CoV (n = 7, 31.8%) and RHV (n = 6, 27.3%), followed by PIV 1–4 (n = 4, 18.2%), RSV (n = 3, 13.6%), ADV (n = 3, 13.6%), influenza A (n = 1, 4.5%), and MPV (n = 1, 4.5%). One person had both CoV and RHV identified. Of the 21 cases, 19 (90.5%) had the virus identified on a lower respiratory sample (bronchoalveolar lavage) and 2 (9.5%) had the virus identified from a nasal wash sample.

3.3. RVI and Development of De Novo Donor-Specific Antibodies

Donor and recipient HLA typing was available for all subjects, and none had DSA detected at baseline. In 6/21 (29%) of the cases and 1/21 (5%) of the controls, *de novo* class II DSA was identified in the second sample ($p = 0.09$). All Class II DSA were identified at the DQ locus. No new class I DSA was identified among cases or controls. Post hoc analyses were performed on the majority of subjects (34 of 42) using HLA MatchMaker (www.epitopes.net) at the DQ locus; this revealed an average DQ difference of 1.43 in controls and 1.13 in cases, suggesting that cases and controls had similar degrees of HLA-DQ mismatch at baseline.

Figure 1 outlines the breakdown of the new class II DSAs in the cases by RVI type and location (BAL or nasal wash), and the new class II DSA in the control. Figure 2 demonstrates the time between RVI and DSA in six case subjects who developed DSA post-RVI. In these six subjects with *de novo* Class II DSAs, the median time to RVI was 64 days post-transplant (IQR: 50–76 days) and median time from RVI to DSA detection was 124 days after RVI (IQR: 79–163 days). None of the patients who had new DSA had preceding CMV pneumonia or pneumonitis. Similar proportions of patients, with and without DSA, had evidence of rejection prior to the second sample; of the seven patients who developed DSA, three out of seven (42.9%) had acute rejection diagnosed prior to the second sample (two of these were cases and the rejection were diagnosed either after the RVI or concurrently with the RVI, and one was the control). In those who did not develop DSA, 14/35 (40%) had rejection diagnosed prior to the second sample.

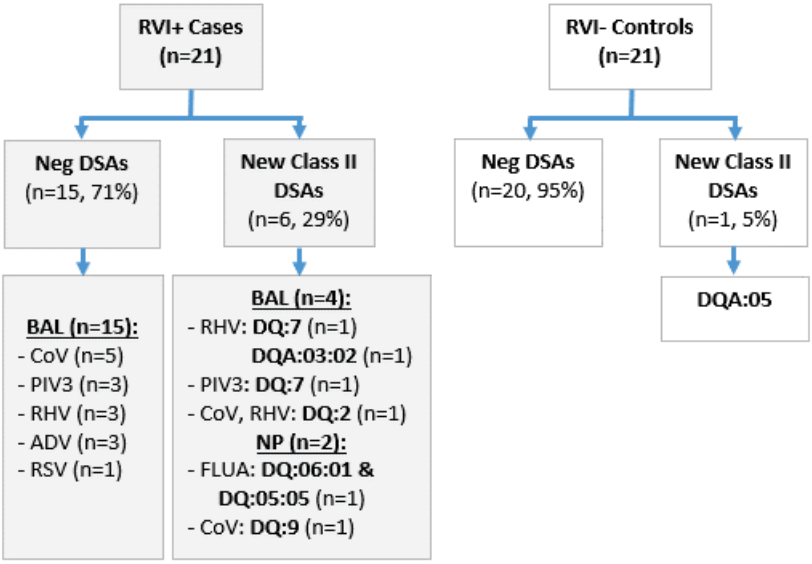


Figure 1. Flow chart depicts development of DSA in cases and controls and characteristics of RVI among cases. BAL, bronchoalveolar lavage. NP, nasopharyngeal swab. CoV, coronavirus (endemic). PIV3, parainfluenza virus 3. RHV, rhinovirus. ADV, adenovirus. RSV, respiratory syncytial virus.

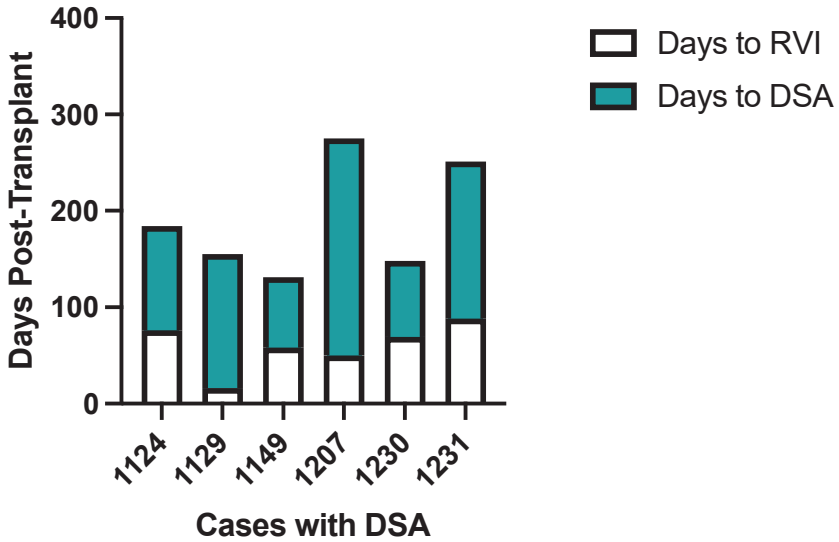
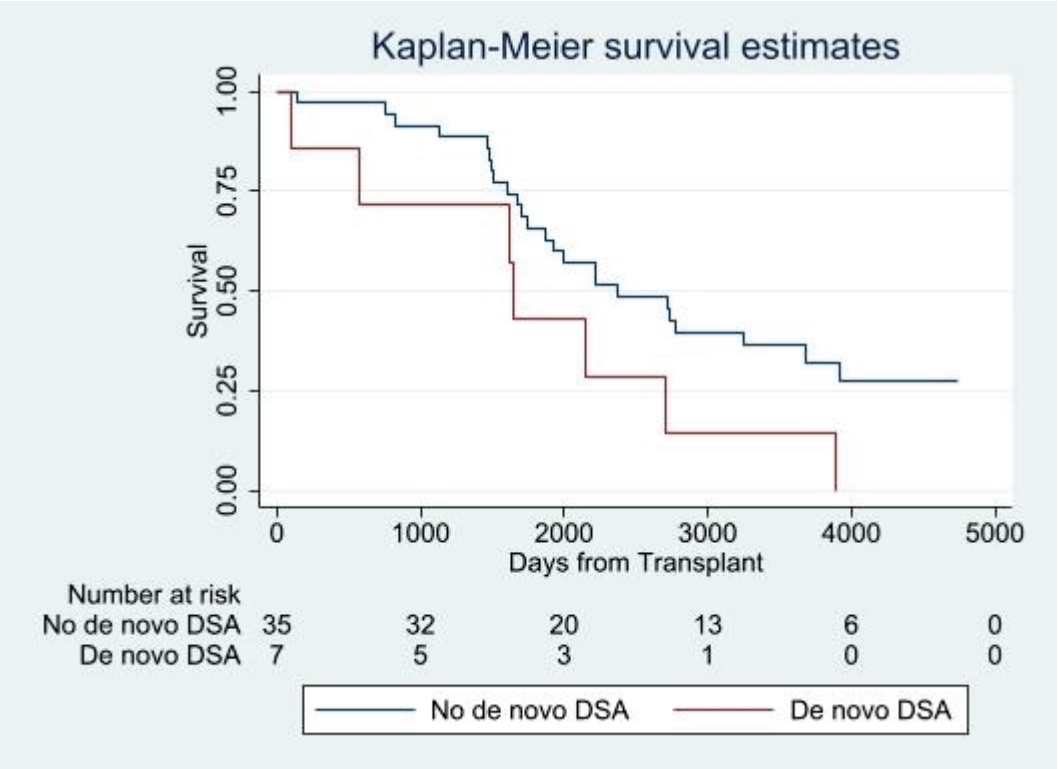


Figure 2. Bar plots show time (days) post-transplant for RVI and DSA development among the 6 cases under study. Days to RVI is indicated in white and days to DSA is indicated in aqua. De-identified case numbers are presented on the x-axis.

3.4. DSA and Development of Chronic Lung Allograft Dysfunction and/or Death

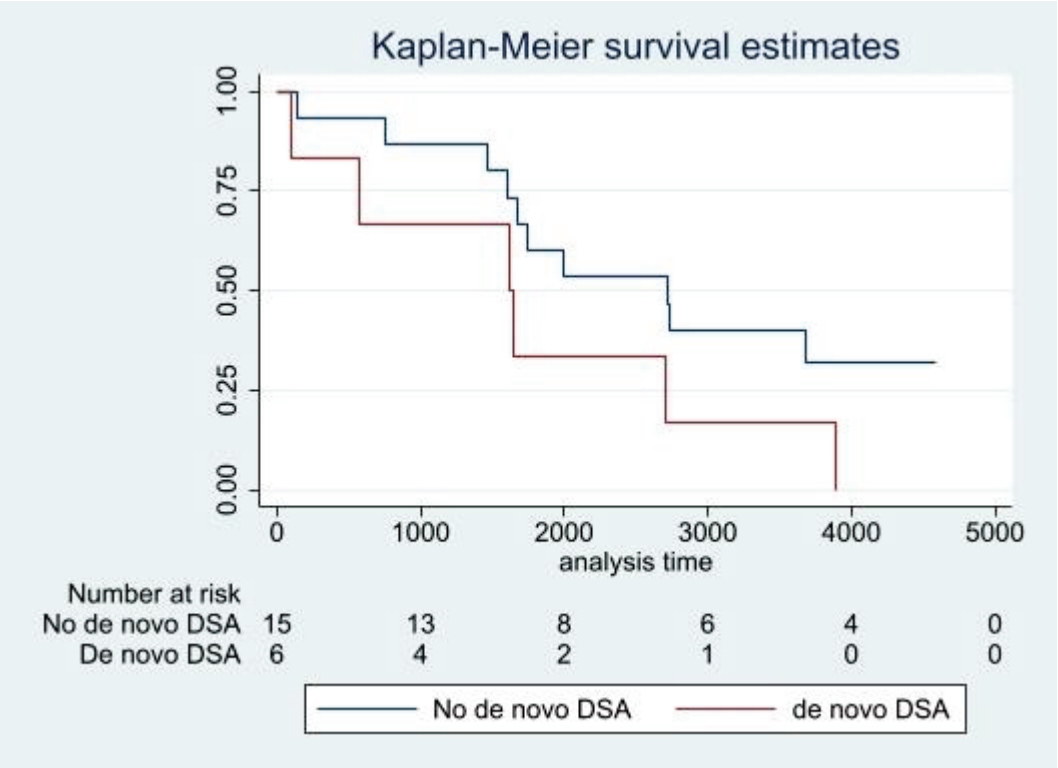
By the end of the follow-up period, 31/42 (73.8%) of the entire LTR cohort (cases and controls) had either developed CLAD (n = 24; 14 BOS, 1 RAS, 6 mixed, 3 undefined) or died prior to CLAD development (n = 7). Median (IQR) time to CLAD or death was 4.8 (4.1–7.4) years. Mortality at the end of the follow-up period (including those who had CLAD and subsequently died) was 22/42 (52.4%). Overall, 7/7 (100%) of the LTRs with *de novo* class II DSA (cases and controls) either developed CLAD or died: 5/7 (71.4%) developed CLAD, 5/7 (71.4%) died (including 3 patients who got CLAD first), and 2/7 (28.6%) died without CLAD. In contrast, among LTRs (cases and controls) who did not develop new class II DSAs, 24/35 (68.6%) either developed CLAD or died: 19/35 (54.3%) developed CLAD, 17/35 (48.6%) died (including 12 patients who got CLAD first), and 5/35 (14.3%) died without CLAD. Figure 3a shows a Kaplan–Meier curve for the composite endpoint of CLAD and death across the entire LTR cohort (cases and controls) who either developed new class II DSAs or did not.

When the analysis was restricted to cases alone (only those with symptomatic RVI), 6/21 (28.6%) had *de novo* DSAs and 15/21 (71.4%) did not. CLAD developed in 14/21 (66.7%) of cases and 10/21 (47.6%) died (8 died after CLAD development). Five out of six (83.3%) cases with *de novo* class II DSAs vs. nine out of fifteen (60%) cases without *de novo* class II DSAs developed CLAD, 4/6 (66.7%) cases with *de novo* class II DSAs vs. 6/15 (40%) cases without *de novo* class II DSAs died, and 6/6 (100%) cases with *de novo* class II DSAs vs. 10/15 (66.7%) cases without *de novo* class II DSAs either developed CLAD or died (Figure 3b).



(**a**)

Figure 3. *Cont.*



(**b**)

Figure 3. (**a**) Kaplan–Maier of composite endpoint of CLAD and death by development of *de novo* DSA. (**b**) Kaplan–Maier of composite endpoint of CLAD and death by development of *de novo* DSAs in LTRs with symptomatic respiratory virus infection (cases).

4. Discussion

In this study, we utilized a single-center cohort of lung transplant recipients with well-characterized respiratory viral infection and adjudicated CLAD to investigate the association between symptomatic RVI and the development of *de novo* DSA. As an exploratory analysis, we also described the long-term development of CLAD and death in LTRs who developed DSAs versus those who did not. We found that LTRs who developed *de novo* DSAs were those with documented prior symptomatic RVI at a frequency that approached statistical significance. Similar to the data reported from other cohorts of LTRs [13–17], we found that the development of *de novo* DSA, and in particular, HLA-DQ, ref. [3] was also associated with the onset of CLAD and death in our cohort.

Previous studies did not find an association between RVI and *de novo* DSA [27,28]. However, these studies used positive viral PCR alone without symptom assessment. Thus, we believe the discrepant findings between these studies and ours may be related to our use of symptomatic RVI as part of the inclusion criteria. We hypothesize that symptomatic versus asymptomatic RVI is more likely to be associated with tissue injury and a cytokine milieu conducive to the development of off-target alloimmune responses that may contribute to CLAD in the lung transplant recipient. For example, as has been described in autoimmunity [29], toll-like receptor and interferon-γ signals elaborated from the lung transplant recipient in response to viral infection may activate bystander pre-formed HLA-reactive memory B cells to differentiate into pathologic HLA-antibody secreting plasma cells. Alternatively, and similar to the off-target effects of viral infection on allo-active T cells, ref. [30] viral infection may induce the expansion of allo-active B cells that cross-react to both viral and HLA epitopes. These data raise the possibility of potential mechanistic linkages among symptomatic RVI, DSA development, and CLAD to be explored in future studies.

Our study has some limitations. First, due to the relatively small patient number in this single-center cohort, we had limited power to detect significant differences and to investigate possible confounders of the strong trend between symptomatic RVI and the development of DSAs. Although we were able to demonstrate similar proportions of acute rejection prior to the second sample in groups with *de novo* DSAs versus those without, we did not have comprehensive data on clinically relevant bacterial pneumonia preceding the development of the DSAs. Future prospective studies should focus on the systematic collection of these potential confounders. Second, we did not have uniform assessments of the RVI episodes, including symptom surveys, duration of viral shedding, or consistent imaging to assess the degree of lower tract disease. Third, we did not assess the development of cellular alloimmunity (e.g., alloreactive T cells) or non-HLA Abs after RVI as a contributing factor to CLAD. Fourth, we did not have allograft biopsies before and after RVI or before and after *de novo* DSAs to directly demonstrate allograft damage from either insult. Finally, our serum biobank was not collected at routine time points after RVI to more precisely define the temporal relationship between RVI and development of *de novo* DSAs.

Our study also had several strengths. We used a well-characterized cohort of LTRs with uniform and immediate post-transplant follow-up, included only symptomatic RVI cases, used newer definitions and an endpoint of adjudicated CLAD as previously described [31], and utilized a control group with closely matched timing of samples post-transplant. We also included an evaluation of cofounders from donor/recipient HLA mismatch and we found the majority of LTRs with (cases) or without (controls) symptomatic RVI did not have significant differences in HLA-DQ loci epitope matching as predicted by HLA MatchMaker (www.epitopes.net). Therefore, we do not believe that the development of *de novo* HLA-DQ DSAs in LTRs with prior symptomatic RVI is due to inherent differences in donor/recipient HLA matching between cases and controls.

Despite the above limitations, we believe that the hypothesis generating findings reported from this retrospective single center LTR cohort remain relevant today. For example, the majority of RVIs that we report here are from endemic coronaviruses with lower respiratory tract tropism. We know that LTRs have worse morbidity and mortality after SARS-CoV2 infection [32], but we do not yet have a complete understanding of the post-acute sequelae of SARS-CoV2 on the development of alloimmunity or long-term lung allograft dysfunction. Bystander tissue-restricted and autoimmune antibodies have been reported in the normal host after SARS-CoV2 infection [33–35], and these findings suggest that similar off-target immune responses may develop after SARS-CoV2 infection in LTRs [36,37]. Therefore, even though this cohort was enrolled prior to the SARS-CoV2 pandemic, these data document a trend toward off-target *de novo* DSA development after RVI from endemic coronaviruses and may possibly be relevant to long-term outcomes of LTR with SARS-CoV2 infection.

In conclusion, the association between symptomatic RVI and CLAD has been reported by us and others [5–17]. Understanding the mechanisms mediating this association is necessary to develop strategies to prolong the life span of the lung allograft. While our data suggest the potential role of *de novo* DSAs in mediating CLAD development after symptomatic RVI, future prospective studies with larger numbers of LTRs and inclusive of LTRs with SARS-CoV2 are needed to confirm and extend these findings.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/v16101574/s1.

Author Contributions: Conceptualization, A.N., C.E.F. and A.P.L.; methodology, A.N., C.E.F. and A.P.L.; formal analysis, C.E.F. and A.N.; investigation, A.N., C.E.F., A.P.L., J.H. and J.T.K.; resources, A.N., C.E.F., J.H. and J.T.K.; writing—original draft preparation, A.N., C.E.F., A.P.L., J.H. and J.T.K.; visualization, A.N. and C.E.F. All authors have read and agreed to the published version of the manuscript.

Funding: AN Grant Support: R21AI152006, U19AI142737, U01AI163072, and U19AI181105. JTK Grant Support: U19AI142737. APL Grant Support: AI163090, AI184205, and HL102547. CEF Grant Support: HL143050 and U01AI163090.

Institutional Review Board Statement: This study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Institutional Review Board of the University of Washington (IRB#44580, date of approval 4 January 2019).

Informed Consent Statement: With the approval of our IRB, written informed consent was not given for the purposes of this study as the samples were part of an existing biorepository and no identifiable information was used.

Data Availability Statement: The raw data supporting the conclusions of this article may be made available by the authors on request.

Conflicts of Interest: The authors declare no conflicts of interest.

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viruses

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Cytokine Dynamics and Herpesvirus Interactions in Pediatric Liver and Kidney Transplant Recipients: The Distinct Behavior of HCMV, HHV6, HHV7 and EBV

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Abstract: Pediatric solid organ transplant (SOT) recipients face a challenging balance between immunosuppression and graft rejection. While Epstein–Barr Virus (EBV) and cytomegalovirus (HCMV) are known contributors to post-transplant lymphoproliferative disease and graft rejection, respectively, the roles of herpesvirus 6 and 7 (HHV6 and HHV7) and the impact of these herpesviruses on cytokine levels remain unclear, leading to gaps in clinical practice. In this associative study, we measured 17 cytokines using a Bio-Plex assay in a meticulously curated plasma sample pool (N = 158) from pediatric kidney and liver transplant recipients over a one-year follow-up period. The samples included virus-negative and virus-positive cases, either individually or in combination, along with episodes of graft rejection. We observed that the elevation of IL-4, IL-8, and IL-10 correlated with graft rejection. These cytokines were elevated in samples where HCMV or HHV6 were detected alone or where EBV and HHV7 were co-detected. Interestingly, latent EBV, when detected independently, exhibited an immunomodulatory effect by downregulating cytokine levels. However, in co-detection scenarios with β-herpesviruses, EBV transitioned to a lytic state, also associating with heightened cytokinemia and graft rejection. These findings highlight the complex interactions between the immune response and herpesviruses in transplant recipients. The study advocates for enhanced monitoring of not only EBV and HCMV but also HHV6 and HHV7, providing valuable insights for improved risk assessment and targeted interventions in pediatric SOT recipients.

Keywords: transplantation; herpesviruses; EBV; graft-rejection; cytokines

1. Introduction

The β-herpesviruses, which include cytomegalovirus (HCMV), human herpesvirus 6A (HHV6A), 6B (HHV6B), and 7 (HHV7), as well as the γ-herpesviruses, Epstein–Barr virus (EBV) and Kaposi sarcoma-associated virus (KSHV), are human viruses known for causing lifelong persistent infections. Particularly, β- and γ-herpesviruses target immune cells for infection, establishing host cell lifelong reservoirs in differentiated lymphoid and myeloid cells or hematopoietic progenitors.

A significant proportion of the global population carries at least three of these herpesviruses, which is a prevalence largely attributed to their biphasic life cycle, encompassing latent and lytic phases. The latent phase is characterized by low to no expression of viral genes, enabling evasion of the immune system and persistence in the host. In contrast, during the lytic phase, there is a heightened expression of viral genes leading to the production of new viral infectious particles [1]. The transition from the latent to the lytic

phase is known as reactivation. While the majority of infected hosts remain asymptomatic, in cases of associated diseases, the reactivation of these herpesviruses is typically observed and measured as detectable viral loads in peripheral blood.

In individuals with compromised immune systems, β- and γ-herpesvirus emerge as a substantial contributor to morbidity and mortality, as exemplified in solid organ transplant recipients undergoing pharmacologic immunosuppression. The intersection of the need for immunosuppression, with consequently herpesvirus reactivation, jeopardizes the success of the transplant, leaving patients vulnerable to clinical complications, such as organ rejection, post-transplant lymphoproliferative syndrome (PTLD) and HCMV-disease [2–6]. This delicate balance requires optimal clinical management with a focus on the monitoring of viral loads and the implementation of preemptive strategies to mitigate the impact of the herpesviruses.

In addition to their individual effects, the simultaneous detection of multiple herpesviruses has been reported in transplanted patients with some studies supporting an association between co-detection and worse clinical outcomes [7]. The tropism for immune cells and reliance on similar mechanisms for reactivation support the notion that β- and γ-herpesviruses mutually influence their biological cycles, collectively impacting the survival of the transplanted organ and the transplanted patient [7]. Cytokines are likely important mediators of mutual connection, since all β- and γ-herpesviruses have evolved immunomodulatory genes not only to block antagonizing host responses but also to influence the activation, survival, differentiation, and expansion of the immune cells that act as reservoirs for their persistent infections. Furthermore, β- and γ-herpesviruses encode their own set of functional homologs of immune-related genes, including virokines [8–10].

A previous analysis of this pediatric cohort revealed associations between specific herpesviruses and rejection. Notably, HCMV and HHV6 alone appeared to be associated with rejection, while EBV contributed to rejection in co-detection events, suggesting interactions between EBV and the β-herpesviruses. In this study, searching for potential channels of herpesvirus interactions, we analyzed the concentrations of 17 cytokines in the plasma of post-transplant patients over a one-year follow-up period. We correlated these cytokine concentrations with the loads of β- and γ-herpesviruses and with graft rejection. This investigation aims to deepen our understanding of the intricate relationship between immunosuppression, herpesvirus infection, and clinical outcomes in solid organ transplant recipients.

2. Materials and Methods

2.1. Patients and Clinical Samples

We collected a total of 158 blood samples from a cohort comprising 20 pediatric patients who underwent liver or kidney organ transplantation. These samples were carefully selected from a larger pool of 495 samples gathered over a one-year follow-up period, involving 34 post-transplant patients, 22 with renal and 12 with liver transplantation [11]. During the initial three months post-transplantation, we collected blood samples every two weeks, reducing the frequency to once per month thereafter. In a prior report, we detailed the viral DNAemia of β-herpesvirus and EBV in the leukocyte and plasma fraction of the 495 blood samples [11]. Throughout the follow-up period, ten patients experienced episodes of acute graft rejection, equivalent to 17 rejection samples, with 82% of these rejection episodes coinciding with an episode of viremia. No episode of PTLD was reported in this cohort during the follow-up. Our qPCR does not distinguish between HHV6A and HHV6B, and we will be collectively referring to these viruses as HHV6. We also analyzed the presence of KSHV, but no sample was positive for KSHV infection/detection. In the Results section, see Figure 1a for a flow chart of the samples taken for cytokine analysis; Appendix A, Figure A1 for the timeline of data collection illustrating the timepoints of viral positivity and graft rejection; and Appendix A, Table A1 for the EBV and HCMV donor and recipient serology.

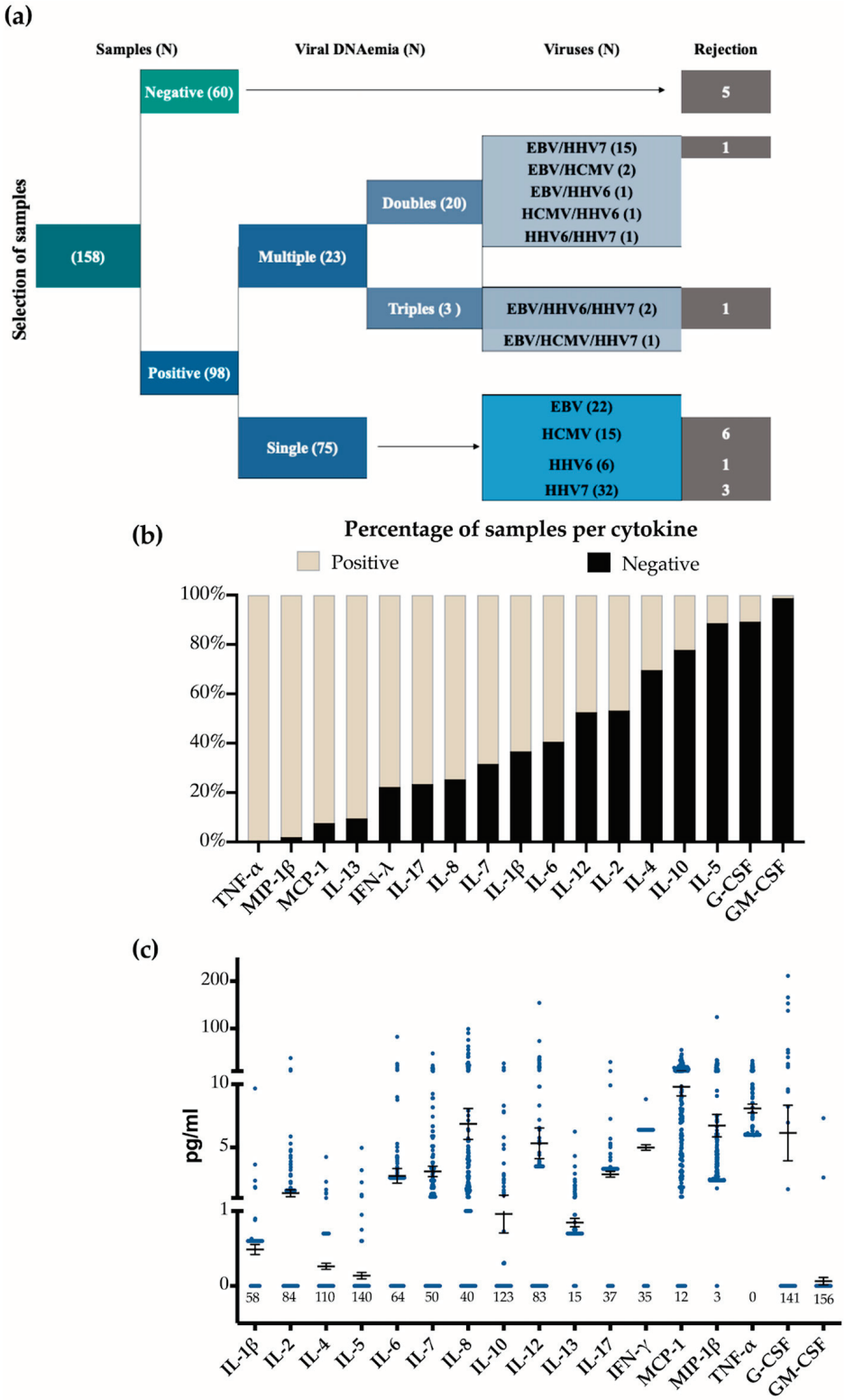

Figure 1. Selection of samples for cytokine analysis. (**a**) Depiction of the plasma samples analyzed. The Venn diagram shows the positive DNAemia by virus. Detection of viruses in the original cohort was as follows: HHV7 = 39%, EBV = 30%, HCMV = 20% and HHV6 = 11%. We tried to preserve this proportions in this subset of samples. (**b**) Percentage of positive (beige) and negative (black) samples for each cytokine analyzed. The numbers below are the cytokine negative samples. (**c**) Concentration for each cytokine among samples showing the mean and standard error.

As stated in our previous publication, this study was approved by the Ethical, Biosecurity and Scientific review boards of the Children's Hospital of Mexico Federico Gómez (Registry HIM-2016-021). Graft rejection was diagnosed from clinical, laboratory and histopathological data, following the Banff global consensus classification. Prior to sample collection, patients and their parents/guardians were informed about the nature of the study, and those who were willing to participate signed a letter of consent (parents/guardians) and a letter of assent (children older than 10 years). Children with incomplete follow-up or suffering hyperacute graft rejection the first days after transplantation were excluded from the study. All enrolled patients were treated according to the ethical guidelines of our institution [11].

2.2. Immunoassay

We utilized 200 μL of plasma to determine the presence and concentration of cytokines IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13, IL-17A, IFN-γ (interferon-γ), TNFα (tumor necrosis factor-α), MCP-1 (monocyte chemoattractant protein 1), MIP1-β (macrophage inflammatory protein 1β), G-CSF (granulocyte-colony stimulating factor) and GM-CSF (granulocyte/macrophage-colony stimulating factor) through multiplex immunoassays. We used the kit Bio-Plex Pro™ Human Cytokine 17-plex (BioRad, Hercules, CA, USA) and the Bio-Plex 200 Systems, following the manufacturer's instructions throughout the entire process.

2.3. Viral Detection in Clinical Samples

Viral detection was performed using an in-house multiplex qPCR that simultaneously detects and quantifies beta and gamma human herpesviruses as previously reported [11]. In this earlier study, blood samples were fractionated into cellular components and plasma, and detection was carried out in both compartments. Since herpesviruses exhibit a bipartite life cycle oscillating between latent and lytic phases, viral loads found in the cell fraction were considered more indicative of latency, while viral loads found in plasma were considered more indicative of an active lytic cycle. It is important to highlight that before the extraction of plasma DNA, a DNase treatment step was performed to avoid quantifying viral DNA from broken cells.

2.4. Statistical Analysis

As we did not observe a significant difference in cytokine levels between patients who underwent kidney or liver transplants, we combined all data for statistical robustness. Forty-six percent of the cytokine-positive samples were below their technical limit of detection (LOD); to facilitate the statistical analyze of these cytokines, we used the substitution method to maintain statistical rigor [12]. This method allows the analysis of data below the LOD, which is also called censored data. The most common and easiest strategy is a simple substitution in which censored values are either replaced by zero, replaced by a fraction of the detection limit (usually $1/2$ or $1/\sqrt{2}$), or replaced with the LOD itself. After testing the substitution with zero and the LOD, and finding no differences, we opted to use each cytokine's specific LOD to substitute the censored data (Table 1). Using substitution methods for values below the LOD can potentially distort estimates and statistical tests, particularly for cytokines with an elevated proportion of samples under the LOD. While we believe our approach is sound and follows established practices, we acknowledge this potential limitation.

Using Mann–Whitney t-tests, we compared the concentrations for each cytokine between groups, for instance, samples with positive viral DNAemia versus those negative for viral DNAemia; or samples positive for rejection versus those negative for rejection. We used the Kruskal–Wallis test to compare the cytokine concentrations among multiple groups, such as multiple, single and no viral DNAemia. Outliers were removed using a ROUT test with a Q = 1% in all comparative analyses (Figure A2). Correlation analyses were performed using Spearman tests.

Table 1. Limit of detection values for all cytokines tested.

Cytokine	LOD *	Cytokine	LOD *	Cytokine	LOD *	Cytokine	LOD *
IL-1β	0.6	IL-7	1.1	IL-17	3.3	GM-CSF	1.7
IL-2	1.6	IL-8	1	IFN-γ	6.4	G-CSF	2.2
IL-4	0.7	IL-10	0.3	MCP1	1.1		
IL-5	0.6	IL-12	3.5	MIP1β	2.4		
IL-6	2.6	IL-13	0.7	TNF-α	6		

* LODs: limits of detection in pgs/mL.

We employed relative risk (RR) analyses to quantify the association between the presence of specific cytokines and the outcomes of interest, namely rejection and the detection of herpesviruses. The RR provides a measure of the strength of association between an exposure (in this case, cytokines or viral DNAemia) and an outcome (rejection or presence of a herpesvirus) (Appendix B).

Heat maps were generated to illustrate the disparities between the means of the analyzed groups. These differences were normalized to percentages, and the colorimetric scale was adjusted to reflect negative or downregulated values in blue, while upregulated or positive values were represented in yellow. This color scheme was in reference to values found in the samples negative to the variables analyzed, serving as the basal reference. We used GraphPad Prism 9 software to construct graphs and visualize data.

3. Results

3.1. TNF-α, MIP-1β, MCP-1 and IL-13 Are Elevated in the Plasma of Post-Transplant Patients

We carefully selected 158 blood samples from a larger cohort of pediatric patients who underwent liver or kidney organ transplantation (Figure 1a). The samples selected included all different variables mirroring the proportions found in the complete cohort: 98 tested positive for viral DNAemia of at least one of the herpesviruses, which is suggestive of exacerbated infection. Within this group, 23 exhibited a co-detection of multiple herpesviruses, while 75 samples showed single-virus positivity. The remaining 60 samples tested were negative for viral DNAemia. We also included in our analysis 17 samples collected during acute rejection episodes, of which five were negative to virus detection, and 12 coincided with the detection of one or more of the herpesviruses, while 55 samples were negative to rejection and viral DNAemia. All rejection episodes were T cell mediated, and graft rejection was diagnosed according to the Banff global consensus classification [13]. Table 2 shows the demographic and clinical data of this subset of patients.

Table 2. Patients' clinical and demographic data.

Transplant	Renal	Liver
N° of patients	13	7
Age range at transplant (median)	6–17 years (14.5)	4–8 years (4.5)
Sex		
Female	15%	57%
Male	85%	43%
Type of donor		
Diseased	54%	86%
Living	46%	14%
N° of samples (median/patient)	192 (15)	92 (13)
Pretransplant diagnosis	77% ESRD * of unknown etiology	14% Bile duct atresia 14% Fulminant Hepatitis
	8% Focal and segmental glomerulosclerosis	14% Neonatal giant cell hepatitis 14% Tyrosinemia
	8% ESRD secondary to **JRA hypoplasia	14% Bayler disease 14% Alalgille syndrome
	7% Microscopic polyangiitis	14% Progressive intrahepatic family cholestasis

* ESRD: end-stage chronic kidney disease; ** JRA: juvenile rheumatoid arthritis.

We measured 17 different cytokines in the plasma of the selected samples; each of these samples tested positive for at least one of the cytokines. Figure 1b and Table 3 show the number of samples that tested positive and negative for each cytokine. TNF-α, MIP-1β, MCP-1, and IL-13 were detected in more than 90% of the samples, IFN-γ, IL-17, IL-8, IL-7, IL-1β, and IL-6 were positive in approximately 50% to 80% of the samples, and the remaining cytokines were found in fewer than 50% of the samples. Figure 1c shows the values found for each cytokine. Table S1 shows all the values found for cytokine detection and viral DNAemia in all analyzed samples.

Table 3. Positive and negative samples for each cytokine.

Cytokine	Positive	Negative	Cytokine	Positive	Negative
TNF-α	158	0	IL-6	94	64
MIP-1β	155	3	IL-12	75	83
MCP-1	146	12	IL-2	74	84
IL-13	143	15	IL-4	48	110
IFN-γ	123	35	IL-10	35	123
IL-17	121	37	IL-5	18	140
IL-8	118	40	G-CSF	17	141
IL-7	108	50	GM-CSF	2	156
IL-1β	100	58			

All cytokines were positive for at least 2 samples.

3.2. Elevated Cytokines Are Preferentially Found in Samples with Multiple Viral Detection

We compared cytokine concentrations in samples with single viral DNAemia, multiple viral DNAemia, and without DNAemia. Significant increases in the levels of TNF-α, IFN-γ, IL-17, IL-12, IL-8, IL-7, IL-2, IL-1β, IL-4, and IL-10 were observed in samples with viral detection, whether single or multiple, compared with those without viral detection. Generally, greater increases in cytokine levels were seen in samples positive for more than one herpesvirus, except for IL-7, IL-17, and IFN-γ, which were more elevated in samples with single viral DNAemia. IL-4 and IL-10 were only detected in samples with multiple DNAemia and were never detected in virus-negative or single-virus detection cases (Figure 2a). Cytokines MIP-1β, IL-13, IL-6, IL-5, G-CSF, and GM-CSF did not show any differences between sample groups and are therefore not presented.

We conducted a qualitative risk analysis considering only the frequencies at which each cytokine tested positive or negative in relation to viral DNAemia. Our results indicated that cytokines IL-2, IFN-γ, IL-10, IL-7, IL-12, and IL-17 were 1.6 to 2.8 times more frequently detected in samples with positive viral DNAemia, with a higher representation observed in cases with multiple viral DNAemia, which is consistent with the quantitative analysis (Figure 2b). Figure 2c presents a Venn diagram to summarize the quantitative and qualitative results.

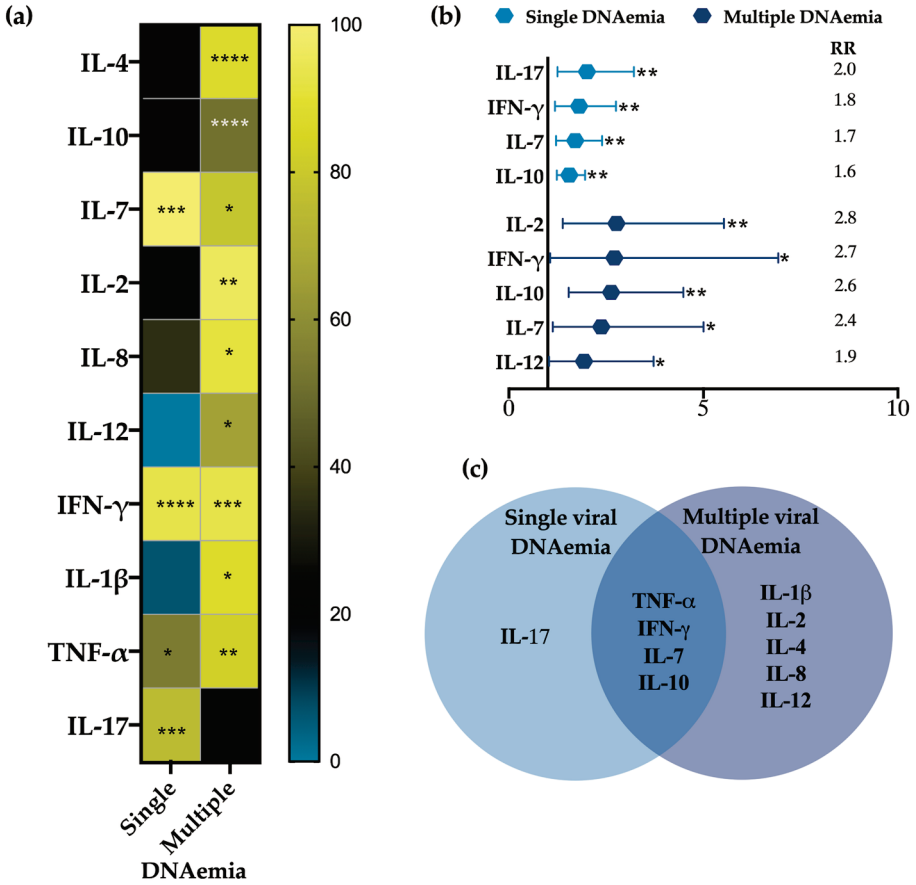

Figure 2. Comparison of the cytokine concentration in samples with or without viral DNAemia. (**a**) Heat map displaying the percentage increase in cytokine concentrations observed in samples with single and multiple DNAemia compared with samples negative for viral detection. The scale of cytokine expression change in percentages is shown on the right. (**b**) Forest plot representing a qualitative analysis of relative risk for cytokine positivity in single and multiple viral DNAemia samples. (**c**) Venn diagram summarizing the cytokines that exhibit differential increases in both single and multiple viral DNAemia samples. Significant values * $p < 0.1$, ** $p < 0.01$, *** $p < 0.001$ and **** $p < 0.0001$.

3.3. Elevated Cytokines Levels Correlate with Detection of the β-Herpesviruses

We assessed which cytokines were elevated in samples positive for each virus both in single and multiple detection events (Figure 3a). Notably, in the case of HCMV and HHV6, cytokine levels were increased in both conditions. In contrast, for EBV and HHV7, most cytokines were elevated when these viruses were co-detected with other herpesviruses. These findings suggest that HCMV and HHV6 alone can lead to high cytokine levels, whereas EBV and HHV7 appear to rely on co-detection with other herpesviruses.

To explore the connection between viral loads and cytokine concentrations, we conducted a Spearman correlation test. We observed significant but generally low to moderate positive correlations, ranging from 0.16 to 0.45 (Figure 3b). Specifically, we observed a significant correlation between the concentration of two cytokines and EBV load, four cytokines and HHV6 load, and six cytokines and either HCMV or HHV7 loads. Collectively, our findings support the notion that the detection of herpesviruses is associated with cytokine levels in post-transplant patients, particularly when multiple herpesviruses are detected. Notably, the β-herpesviruses (HCMV, HHV6 and HHV7) seem to exert a more potent influence on cytokine levels in these patients.

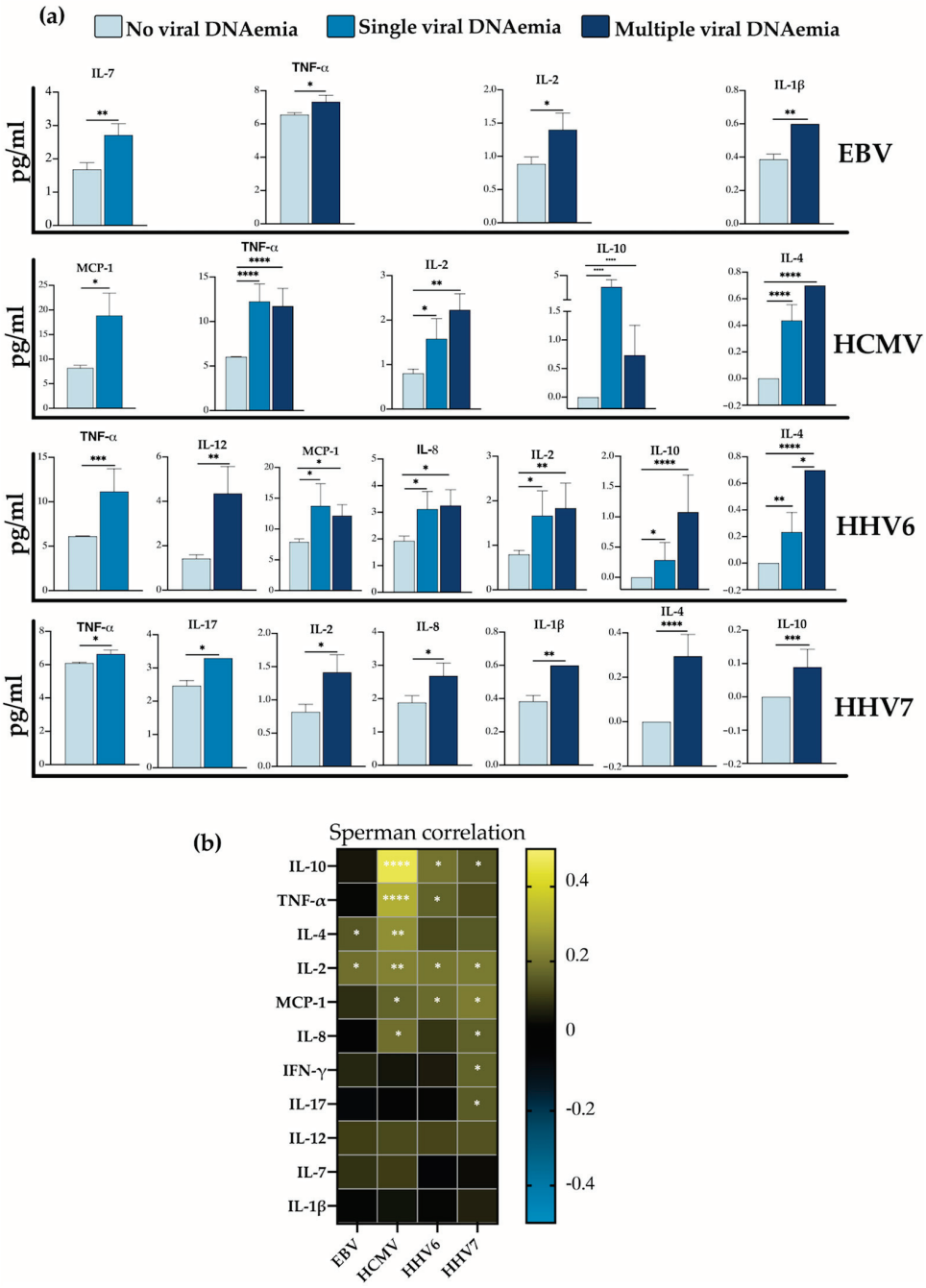

Figure 3. Virus-specific association with elevated cytokines. (**a**) Bar graphs, representing comparisons of the concentrations of each cytokine, between viral DNAemia-negative samples vs. samples with single and multiple viral DNAemia by virus. (**b**) Correlation matrix between the loads of each virus analyzed and the concentration of each cytokine. Significant values * $p < 0.1$, ** $p < 0.01$, *** $p < 0.001$ and **** $p < 0.0001$.

3.4. Cytokines IL-4, IL-8 and IL-10 Significantly Increase in Patients with Graft Rejection

In our previous report, we established an association between herpesvirus DNAemia and graft rejection in this post-transplant patient cohort [11]. We identified two viruses in single-detection (HCMV and HHV6) and two mixes of co-detected viruses (EBV/HHV7 and EBV/HHV6/HHV7) associated with graft rejection. In this study, we aimed to explore whether, within the studied cytokines, we could identify an association with the herpesviruses and/or with graft rejection. We first compiled the cytokines significantly elevated in the four conditions described above: HCMV and HHV6 in single DNAemia and

EBV/HHV7 and EBV/HHV6/HHV7 co-detections (Figure 4a). Various cytokine patterns were observed; for instance, IL-2 was upregulated in all four viral detection conditions, while IL-4, IL-10, MCP-1, and TNF-α were upregulated when HCMV or HHV6 were single-detected. Conditions of EBV/HHV7 and EBV/HHV6/HHV7 co-detection did not exclusively share any cytokine, but HHV6 and EBV/HHV7 shared IL-8 upregulation.

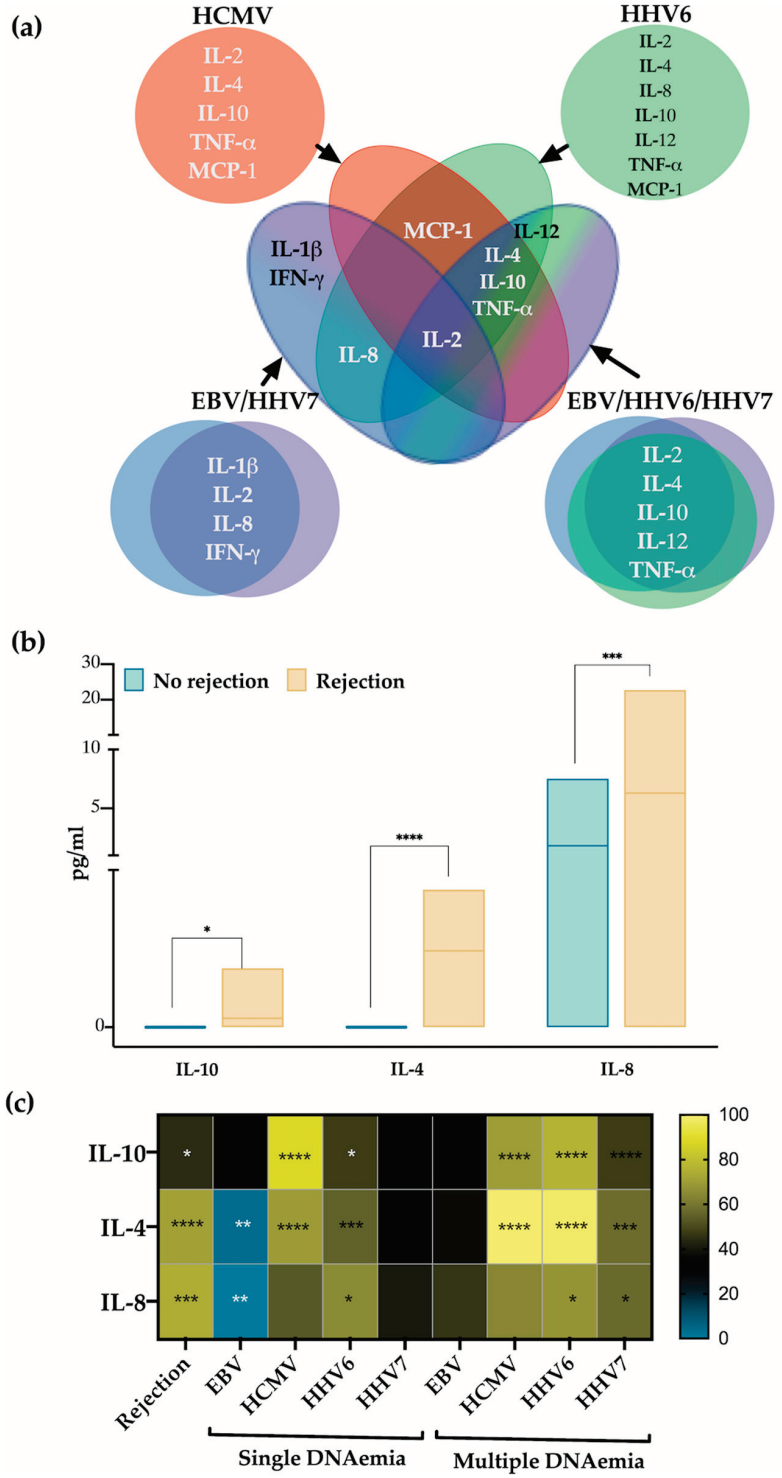

Figure 4. Differentially upregulated cytokines in rejection. (**a**) Venn diagram summarizes the differentially upregulated cytokines in samples with viral DNAemia by virus. (**b**) Bar graph showing the upregulated cytokines in rejection-positive samples. Kruskal–Wallis nonparametric test. (**c**) Heat map representing the comparison of cytokines between samples without viral DNAemia vs. column

1—rejection positive samples, 2—samples positive only for EBV, 3—samples positive only for HCMV, 4—samples positive only for HHV6, 5—samples positive only for HHV7, 6—samples positive for EBV plus other herpesvirus(es), 7—samples positive for HCMV plus other herpesvirus(es), 8—samples positive for HHV6 plus other herpesvirus(es), 9—samples positive for HHV7 plus other herpesvirus(es). Significant values * $p < 0.1$, ** $p < 0.01$, *** $p < 0.001$ and **** $p < 0.0001$.

We conducted a quantitative analysis to compare cytokine concentrations in samples associated with rejection to those without rejection. This analysis revealed a significant increase in the levels of IL-4, IL-8, and IL-10 in the rejection-positive samples (Figure 4b). We created a heat-map of the concentrations of these three cytokines with respect to the herpesviruses either in single or multiple detection (Figure 4c). IL-4 and IL-10 were consistently elevated in samples with HCMV and HHV6 detection: both alone or multiple. HHV7-positive samples also had elevated levels of these cytokines but only in multiple detection. IL-8 was elevated in samples where HHV6 was single-detected or in multiple detection events of both HHV6 and HHV7. On the contrary, EBV appeared to downregulate IL-4 and IL-8 as single detection. The analysis displayed in Figure 4c aligns with the Venn diagrams of Figure 4a, implying a strong correlation between HCMV and HHV6 detection with the upregulation of IL-4 and IL-10, and with HHV6 also correlating with IL-8. Meanwhile, detection conditions that do not involve any of these viruses (conditions with elevated EBV and HHV7) only relate to transplant rejection through IL-8. These findings suggest a role for these cytokines as mediators of virus-induced graft rejection following transplantation, with the β-herpesviruses, particularly HCMV and HHV6, identified as the main triggers of their upregulation.

3.5. Detection of EBV Associates with an Immunomodulatory Effect

A comparison of cytokine concentrations across the different sample groups found that MCP-1, TNF-α, IL-12, IL-8, IL-6, IL-2, and IL-4 exhibited notably lower levels in samples with single EBV detection compared with samples where no herpesvirus was detected (Figure 5a). These findings suggest a potential immunomodulatory role for EBV. The only other instance of a cytokine exhibiting decreased concentration in samples with a single DNAemia was IL-6 and HHV7 (Figure 5a). To visualize the normalized mean difference in percent in cytokine concentrations, we generated a heat map comparing samples where herpesviruses were not detected to those with sole EBV detection (Figure 5b). The heat map illustrates how this immunomodulatory pattern is disrupted when other herpesviruses are co-detected alongside EBV, leading to increased cytokine concentrations across the board.

To provide further insights between the link of EBV detection and cytokines levels, we conducted a qualitative risk analysis similar to the one presented in Figure 2c. This analysis revealed that MCP-1, IL-12, IL-8, MIP-1β, IL-6, IL-2, IL-10, and IL-4 were less frequently observed in samples with EBV-positive single viral DNAemia compared with samples without viral detection (Figure 5c). Therefore, both the qualitative and quantitative analyses support an immunomodulatory role for EBV with both analyses showing high correlation. The sole other exception of a cytokine showing significantly lower levels was IL-5 in single- versus no-HHV7 detection (Figure 5c). However, HHV7 lacked consistency between the quantitative and qualitative risk analyses.

3.6. The β-Herpesviruses Appear to Reverse the EBV Immunomodulatory Effect

We tested the hypothesis that the β-herpesviruses may trigger the reactivation of EBV. In our previous analysis [11], we separated blood samples into cell and plasma fractions, reasoning that EBV detection in the cell fraction would be indicative of latent infection, while detection in the plasma fraction would be indicative of lytic infection and, consequently, of viral reactivation. We explored whether the detection of other herpesviruses alongside EBV altered the fraction where EBV was detected and whether this switch influenced the levels of the cytokines under study. This analysis aimed to provide context for understanding why EBV detection alone was associated with low cytokine levels, whereas this effect changed in cases of multiple viral detections.

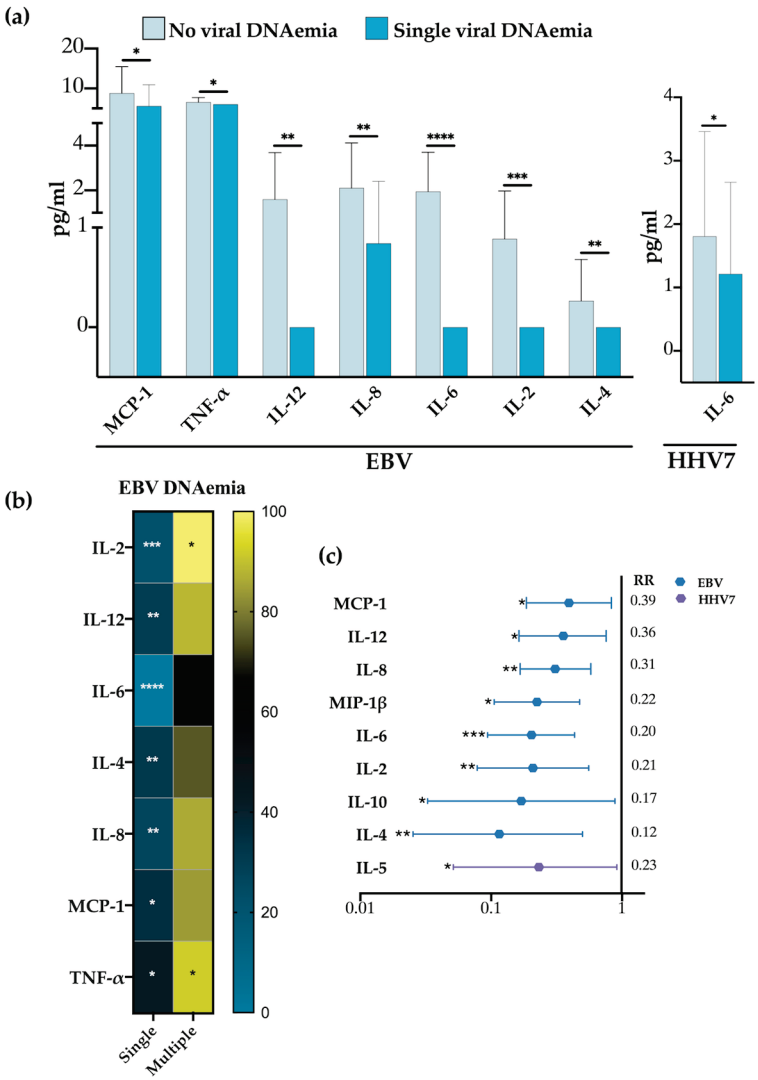

Figure 5. Downregulation of cytokine levels by EBV when comparing viral DNAemia-negative vs. viral DNAemia-positive samples. (**a**) Bar graph showing those cytokines decreased when comparing their concentrations between EBV DNAemia-negative vs. single EBV DNAemia samples. (**b**) Heat map showing the percentage of decrease observed between cytokine concentrations in the negative vs. positive samples both in single and multiple DNAemia. The downregulated and upregulated cytokines are shown in blue and yellow, respectively. (**c**) Forest plot of a relative risk qualitative analysis representing the protective effect yielded by EBV DNAemia. Significant values * $p < 0.1$, ** $p < 0.01$, *** $p < 0.001$ and **** $p < 0.0001$.

We proceeded to analyze the patterns of cytokine concentration in patient samples, categorizing them into four groups: (1) samples with exclusive (single) EBV detection, (2) samples with EBV exclusively detected in leukocytes (suggestive of a latent state), (3) samples with co-detection of EBV and other β-herpesviruses (multiple), and (4) samples with EBV exclusively detected in plasma (suggestive of viral reactivation). As illustrated in Figure 6a, the heat map illustrates that cytokine concentration patterns are similar between groups 1 and 2 as well as between groups 3 and 4. These patterns align with low cytokine levels in the former two groups and high levels in the latter two groups. Linear correlation analysis confirmed this observation, revealing a strong positive correlation between groups 1 and 2 (r = 0.934; $p < 0.0001$) and between groups 3 and 4 (r = 0.7527; $p = 0.0042$) (Figure 6b). Conversely, correlations between groups 1 or 2 versus groups 3 or 4 were all negative (Figure 6c and Table 4).

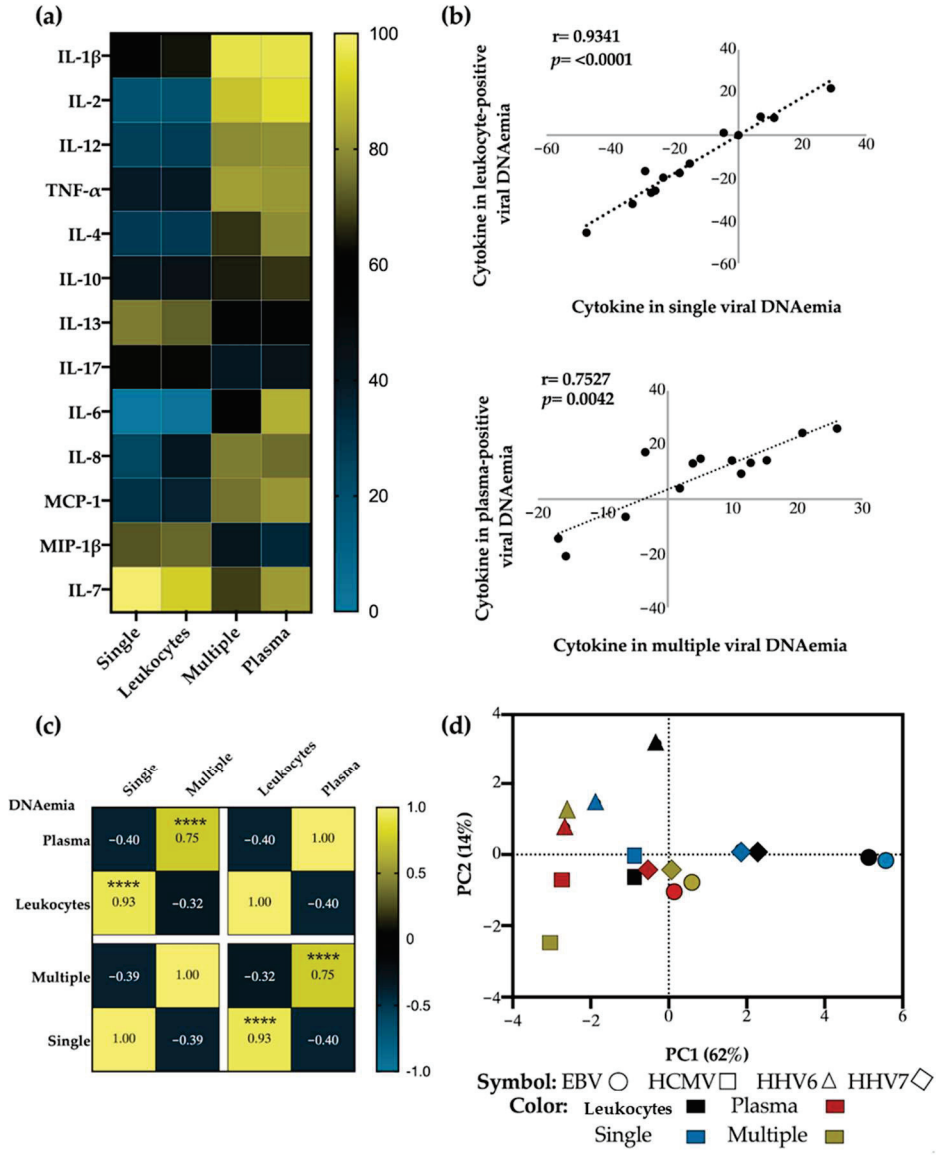

Figure 6. Correlation analysis of EBV detection and cytokine concentration. (**a**) Heat map representing the comparison of cytokines between samples without viral DNAemia versus four groups: column (1) EBV positive samples in single detection; (2) EBV positive samples detected in the leukocyte fraction, (3) EBV positive samples in co-detection with other herpesvirus, and (4) EBV positive samples detected in the plasma fraction. The increase in cytokines is shown in yellow and the decrease in blue. (**b**) Linear regression plots showing the correlation between cytokines levels present in samples with single viral DNAemia versus viral DNAemia detected in leukocytes (**upper panel**), and cytokines in multiple viral DNAemia versus viral DNAemia in plasma (**lower panel**). (**c**) Spearman correlation matrix showing the correlative indexes between the four groups analyzed. (**d**) Principal component analysis showing clusters of groups 1–4 for each herpesvirus analyzed. Significant values **** $p < 0.0001$.

We conducted a similar analysis to the one performed for EBV with the β-herpesviruses. Interestingly, we observed with the β-herpesviruses a distinct pattern to the one observed for EBV. In the case of HCMV and HHV6, we noted positive and significant associations with cytokine concentrations regardless of the blood fraction or the presence of other viruses (Appendix A Figures A2 and A3), in contrast to EBV, which showed negative correlations in the comparisons between groups: single versus plasma and multiple versus leukocytes. Therefore, only EBV in single DNAemia was associated with the downreg-

ulation of cytokine levels when viral loads were exclusively detected in the cell fraction (Table 4 provides all the statistical values). On the contrary, HCMV and HHV6 detection consistently led to heightened cytokine levels in both cellular expansions and viral reactivation as well as in single- and co-detection with other herpesviruses. HHV7 was the only virus with non-significant correlations, with only a close to significant correlation for multiple detection vs. plasma ($p = 0.557$) (Appendix A Figure A4).

Table 4. Summary of the statistical values of the correlation analysis.

Virus	Comparison	r Value	*p* Value
EBV	Single (G1) vs. leukocyte (G2)	0.93	<0.0001
	Multiple (G3) vs. plasma (G4)	0.75	0.0042
	Single (G1) vs. plasma (G4)	−0.4	0.1822
	Multiple (G3) vs. leukocyte (G2)	−0.32	0.2800
HCMV	Single (G1) vs. leukocyte (G2)	0.82	0.0009
	Multiple (G3) vs. plasma (G4)	0.60	0.0320
	Single (G1) vs. plasma (G4)	0.88	0.0001
	Multiple (G3) vs. leukocyte (G2)	0.8	0.0018
HHV6	Single (G1) vs. leukocyte (G2)	0.64	0.0207
	Multiple (G3) vs. plasma (G4)	0.61	0.0302
	Single (G1) vs. plasma (G4)	0.85	0.0005
	Multiple (G3) vs. leukocyte (G2)	0.78	0.0025
HHV7	Single (G1) vs. leukocyte (G2)	0.33	0.2634
	Multiple (G3) vs. plasma (G4)	0.55	0.0557
	Single (G1) vs. plasma (G4)	0.28	0.3502
	Multiple (G3) vs. leukocyte (G2)	0.38	0.1999

Finally, we conducted a comparison of the four groups across all the herpesviruses using principal component analysis (PCA) (Figure 6d, Appendix A Figure A5). Once again, we noted that PC1, which explained the greatest variation in data (62%), distinctly separated EBV groups 1 and 2. EBV groups 3 and 4 appeared to localize closer to any of the other groups, including HCMV and HHV6 groups 1 to 4. The groups closest to EBV groups 1 and 2 were HHV7 groups 1 and 2, while the most distant groups were HCMV and HHV6 groups 3 and 4. This PCA analysis illustrates the unique separation of EBV groups characterized by EBV in single DNAemia, cellular fraction detection, and lower levels of cytokines. The remaining groups are mostly associated with heightened cytokine levels, and all tended to cluster in greater proximity.

Collectively, this analysis supports the idea that when EBV is detected alongside β-herpesviruses, it is in a state of lytic reactivation, whereas it appears to be in a latent state when detected alone. In the latent state, EBV associates with lower cytokine levels, whereas in reactivation, cytokine levels tend to increase. Altogether, these findings suggest that β-herpesviruses can potentially prompt EBV reactivation, leading to a loss of EBV's immunomodulatory capacity.

4. Discussion

β- and γ-herpesviruses have evolved over hundreds of millions of years in close association with our immune system [14]. The capacity to alternate between latent and lytic states endows herpesviruses with a remarkable ability to achieve high fitness within hosts. Despite lifelong persistence, they are generally undetectable, indicating a homeostatic state in which latency is most probably the prevalent viral cycle. However, latency can be dis-

turbed by molecules targeting immune cells, including cytokines, interferons, and bacterial and parasite products that stimulate pattern recognition receptors [15–19]. To modulate the immune system to lessen its antiviral effects and foster viral latency, approximately 30% of the β- and γ-herpesvirus genomes encode proteins or non-coding transcripts that target immune cells or immune-related processes [8,20].

There is evidence suggesting that one herpesvirus can influence the biological cycle of another herpesvirus, although such evidence is limited and has primarily been observed through in vitro experimentation. For instance, HHV6 has been shown to reactivate both EBV and KSHV; HCMV can reactivate KSHV, and HHV7 can reactivate HHV6 [21–24]. In addition to our study [11], multiple other studies support the association of elevated DNAemia of the β- and γ-herpesviruses with post-transplant complications, including EBV and HCMV [25], as well as different combinations of the β-herpesviruses [26–34]. In these studies, the co-detection of more than one herpesvirus is usually associated with a higher risk for an unfavorable clinical outcome. For instance, in a kidney transplant study, the co-detection of EBV and HCMV correlated with graft damage (p = 0.035, RR = 2.1). The detection of HHV6 and/or HHV7 often precedes HCMV detection and HCMV disease [28,34–36], potentially implying cross-reactivation mechanisms. In a solid organ transplant study, HCMV reduced the number of EBV-directed NK cells, increasing the risk of EBV-associated PTLD [37]. However, some studies have not found significant associations between herpesvirus co-detection and enhanced risk for rejection [38,39].

The interactions between herpesviruses appear to be significantly mediated by cytokines. For instance, it has been reported that IL-4, induced by parasites and HSV1, can reactivate γ-herpesviruses [40,41]. In vitro studies have documented that myeloid cells produce IL-1β and TNF-α in response to HHV6 infection [42], and TNF-α induces HCMV reactivation [43]. In this study, we observed an association between the cytokines IL-4, IL-8, and IL-10 with graft rejection with detection of the β-herpesviruses correlating with the upregulation of these cytokines. Notably, HCMV and HHV6 correlated with high cytokine levels whether detected individually or in conjunction with other herpesviruses. In contrast, elevated cytokine levels associated with EBV and HHV7 were predominantly observed when these viruses were co-detected with other herpesviruses. Overall, our findings suggest two potential mechanisms for graft rejection: (i) the sole deregulation of HCMV and HHV6, which alone can lead to high cytokine levels, and (ii) the simultaneous deregulation of EBV and HHV7. This latter mechanism was associated with graft rejection primarily through IL-8.

There is a high heterogeneity in the literature concerning which cytokines are important markers of graft rejection [44]. IL-4 has been associated with liver allograft rejection [45], and the blocking of IL-4 has been proposed to improve long-term grafted kidney preservation [46]. IL-8 has also been related to deterioration of the transplanted liver and proposed as a predictive marker of acute rejection in liver transplantation [47,48]. Several studies have also analyzed IL-10 levels after transplantation, yielding conflicting results. Low levels of IL-10 have been found in chronic kidney rejection [49,50], and IL-10-positive blood cells quantified through ELISPOT were found significantly diminished in acute and chronic kidney rejection [51]. On the contrary, elevated IL-10, IL-17 and IP-10 (interferon gamma-inducible protein 10) had an estimated 94% sensitivity and 97% specificity to predict graft rejection [52]. Elevated levels of IL-10 have also been proposed as a marker for an enhanced risk of HCMV disease in kidney or liver allograft recipients [53]. Importantly, EBV and HCMV secrete BCRF1 and UL111A/cmvIL-10, respectively, which are IL-10 homologous proteins that modulate the host immune system [7]. These viral IL-10s have been shown to inhibit the synthesis of several proinflammatory cytokines, such as IL-1α, IL-6, IL-12, IFN-γ, and TNF-α [9,10].

EBV appeared to display an immunomodulatory capacity, as its single detection was associated with decreased cytokine levels, including those linked with rejection. While there is abundant information about the capacity of EBV to establish an immunosuppressive environment in EBV-associated neoplasms, there is a general lack of information for other

pathological conditions. For instance, EBV can downregulate the capacity of HHV6 to trigger TNF-α secretion in infected blood mononuclear cells [54]. Remarkably, the pattern of cytokine downregulation changed when EBV was co-detected with the β-herpesviruses, leading to elevated levels of the cytokines. A noteworthy observation was that EBV, when detected in isolation, was preferentially found in the cellular fraction of peripheral blood, suggesting a predominant latent state. However, in co-detection with β-herpesviruses, it was found in plasma, suggesting viral reactivation. These observations support a scenario where latent EBV can downregulate cytokine levels, and this balance is disrupted when other β-herpesviruses are upregulated. In such events, EBV is observed in plasma, hinting at a crosstalk between the herpesviruses that may trigger EBV reactivation. Conversely, β-herpesviruses were consistently associated with the upregulation of cytokines in both single and multiple detections as well as in both latent and reactivation states.

An important limitation of this study is that it is an associative study, and in this complex interplay of virus, cytokines and graft rejection, we cannot conclude which one comes first, triggering the others. For instance, IL-10 is considered the prototype of anti-inflammatory cytokines. It is conceivable that IL-10 enhanced levels may arise as a graft-protective mechanism rather than an instrument for graft damage; in other words, the upregulation of IL-10 serves as a compensatory mechanism to counterbalance graft deterioration. In support of this scenario, experimental rat models with an exogenous expression of IL-10 have demonstrated extended graft survival [55–58]. A similar protective role has been proposed for IL-4 in rat experimental models [59,60]. Another limitation of this study is that picks of viral loads and the frequency of simultaneous detections may only be reflective of the degree of host immunosuppression or the donor–receptor previous exposure to the herpesvirus of interest. We were surprised by the low levels of cytokines found in patients, but they may also be reflective of the patients′ immunosuppressive state. Collectively, the limitations of this study underscore the fundamental challenge inherent in observational research: the difficulty of inferring causality from temporal associations alone. While the findings presented here offer valuable insights and generate hypotheses regarding causality, they do not definitively establish causal relationships.

Whether they act as the cause or consequence, our study underscores the importance of monitoring of EBV, HCMV, HHV6, and HHV7, along with IL-4, IL-8, and IL-10, as markers indicating an increased risk of graft rejection during clinical follow-up after transplantation. Regrettably, current worldwide recommendations do not advocate for the consistent monitoring of HHV6 and HHV7, despite HHV6´s strong association with graft rejection. Moreover, there is a need for a standardized method of quantifying herpesviruses to establish clear thresholds of viral loads that strongly indicate unfavorable clinical outcomes. These insights are crucial for advancing our understanding of the significance of herpesvirus detection in clinical outcomes, guiding targeted therapeutic interventions, and developing refined preventive strategies. Ultimately, these efforts aim to improve the overall prognosis and enhance the quality of life for transplanted patients.

5. Conclusions

Latent EBV can downregulate cytokine levels, and this balance is disrupted when other β-herpesviruses are upregulated. In such events, EBV is observed in plasma, hinting at a crosstalk between the herpesviruses that may trigger EBV reactivation. Conversely, β-herpesviruses were consistently associated with the upregulation of cytokines in both single and multiple detections as well as in both latent and reactivation states. Some cytokines like IL-4, IL-8, and IL-10 can act as mediators of virus-induced graft rejection following transplantation, with the β-herpesviruses, particularly HCMV and HHV6, identified as the main triggers of their upregulation.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/v16071067/s1, Table S1: Cytokine concentration and viral load per sample analyzed.

Author Contributions: Conceptualization, A.M.-S. and E.M.F.-P.; Formal analysis, Y.S.-P., J.R.M.-E. and E.M.F.-P.; Funding acquisition, A.M.-S. and E.M.F.-P.; Investigation, Y.S.-P.; Methodology, Y.S.-P., A.M.-S. and J.R.M.-E.; Supervision, A.M.-S. and E.M.F.-P.; Writing—original draft, Y.S.-P. and E.M.F.-P.; Writing—review and editing, A.M.-S., J.R.M.-E. and E.M.F.-P. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by grant CONACyT FOSISS-2015-2-261307 (to Abigail Morales-Sánchez), and Grants HIM/2016/021 and HIM/2019/062 (to Abigail Morales-Sánchez) and HIM/2020/017 (to Ezequiel M. Fuentes-Pananá) from Fondo de Apoyo a la Investigación Hospital Infantil de México "Federico Gómez". The funding institutions had no role in the design and conduct of the study.

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by the Institutional Review Board and Ethics and Biosafety Committees of the Hospital Infantil de México Federico Gómez (protocol Registration HIM/2020/017 with date of approval of 17 June 2020; and protocol Registration HIM/2016/021 with date of approval of 10 December 2015).

Informed Consent Statement: The letters of consent were signed by patients (over 10 years of age) and their parents/guardians.

Data Availability Statement: All relevant data related to this study is presented in main or supplementary figures and tables and in figures and tables of reference 11.

Acknowledgments: Yessica Sánchez-Ponce is a doctoral student from the Programa de Doctorado en Ciencias Biomédicas, Universidad Nacional Autónoma de México (UNAM) and has received CONAHCYT fellowship 704288. We also want to acknowledge the support from Dirección de Investigación from Hospital Infantil de México "Federico Gómez". The authors wish to acknowledge Gustavo Varela Fascinetto and José Carlos Romo Vázquez for their help in forming the original cohort of transplanted patients.

Conflicts of Interest: The authors declare no conflicts of interest. However, the in-house multiplexed qPCR developed to simultaneously analyze EBV, HCMV, HHV6, HHV7 and KSHV has been granted a Mexican (patent MX/a/2017/016321) and USA (U.S. Patent Application No.: 16/772,714) patent entitled "MÉTODO PARA LA DETECCIÓN Y CUANTIFICACIÓN SIMULTÁNEA DE VIRUS DE EPSTEIN-BARR, CITOMEGALOVIRUS, HERPESVIRUS HUMANO 6, HERPESVIRUS HUMANO 7 Y VIRUS DE SARCOMA DE KAPOSI MEDIANTE REACCIÓN EN CADENA DE LA POLIMERASA EN TIEMPO REAL, CUANTITATIVA, MULTIPLEX". The patent is shared by the three authors of the study). This patent has no role in the interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

Abbreviations

EBV	Epstein–Barr virus
G-CSF	granulocyte-colony stimulating factor
GM-CSF	granulocyte/macrophage-colony stimulating factor
HCMV	human cytomegalovirus
HHV6	human herpesvirus 6
HHV7	human herpesvirus 7
IFN-γ	interferon-γ
IL	interleukin
IP-10	interferon gamma-inducible protein 10
KSHV	Kaposi sarcoma associated virus
LOD	limit of detection
MCP-1	monocyte chemoattractant protein 1
MIP1-β	macrophage inflammatory protein 1β
PCA	principal component analysis
PTLD	post-transplant lymphoproliferative syndrome
qPCR	quantitative polymerase chain reaction
RR	relative risk
TNFα	tumor necrosis factor-α

Appendix A

Table A1. EBV and HCMV donor and recipient serology.

Patient ID	EBV Serology		HCMV Serology	
	Donor	**Recipient**	**Donor**	**Recipient**
TR1	Positive	Negative	Negative	Positive
TR4	Positive	No data	Positive	Negative
TR5	No data	No data	Positive	Positive
TR6	No data	No data	Positive	Negative
TR7	No data	No data	Positive	Positive
TR8	No data	No data	Positive	Negative
TR10	Positive	Positive	Positive	Positive
TR13	Positive	Positive	Positive	Positive
TR14	Positive	Positive	Positive	Positive
TR15	No data	No data	No data	Positive
TR16	Positive	Positive	Positive	Positive
TR17	No data	No data	Positive	Positive
TR22	Positive	Positive	Positive	Positive
TH6	Positive	Positive	Positive	Negative
TH7	Positive	Positive	Positive	Positive
TH9	Positive	Negative	Negative	Negative
TH10	Positive	Negative	Positive	Positive
TH12	Negative	Positive	Positive	Positive
TH13	Positive	Positive	Positive	Negative
TH15	Negative	No data	Positive	Negative

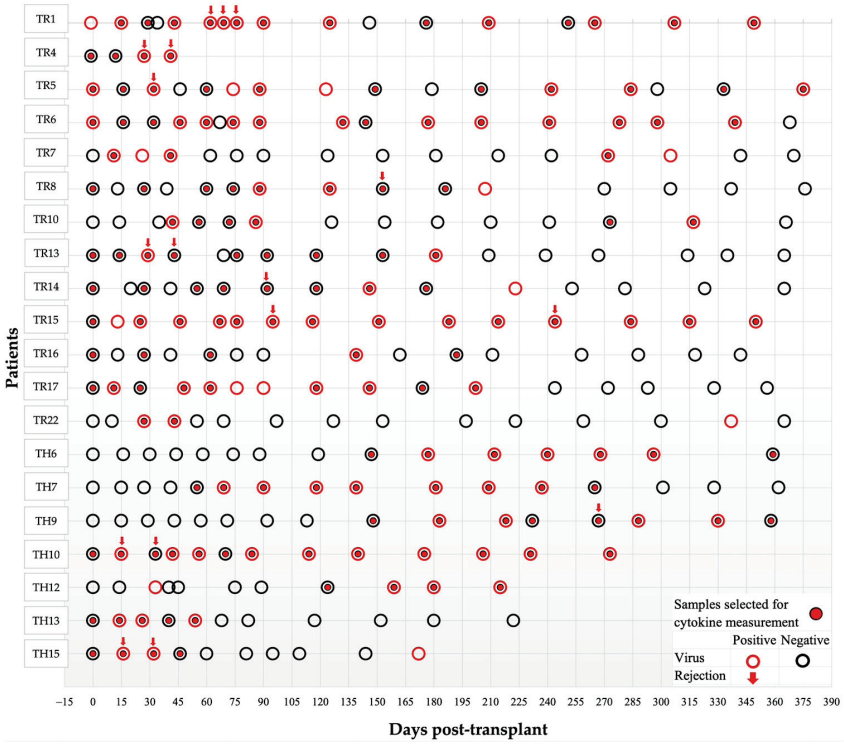

Figure A1. Timeline of the patients' follow-up. The timeline indicates the samples that were positive for viral detection (red circles) and the rejection episodes (red arrows). Samples with a red fill are

those taken for cytokine analysis. All analyzed samples were positive for at least one cytokine. The first 13 patients with code "TR" followed by the patient number (TR1, TR4, TR5, TR6, TR7, TR8, TR10, TR13, TR14, TR15, TR16, TR17 and TR22) were patients who underwent renal transplantation. The last 7 patients with code "TH" followed by the patient number (TH6, TH7, TH9, TH10, TH12, TH13 and TH5) were patients who underwent liver transplantation. In both Table A1 and Figure A1, renal transplant (TR), hepatic transplant (TH).

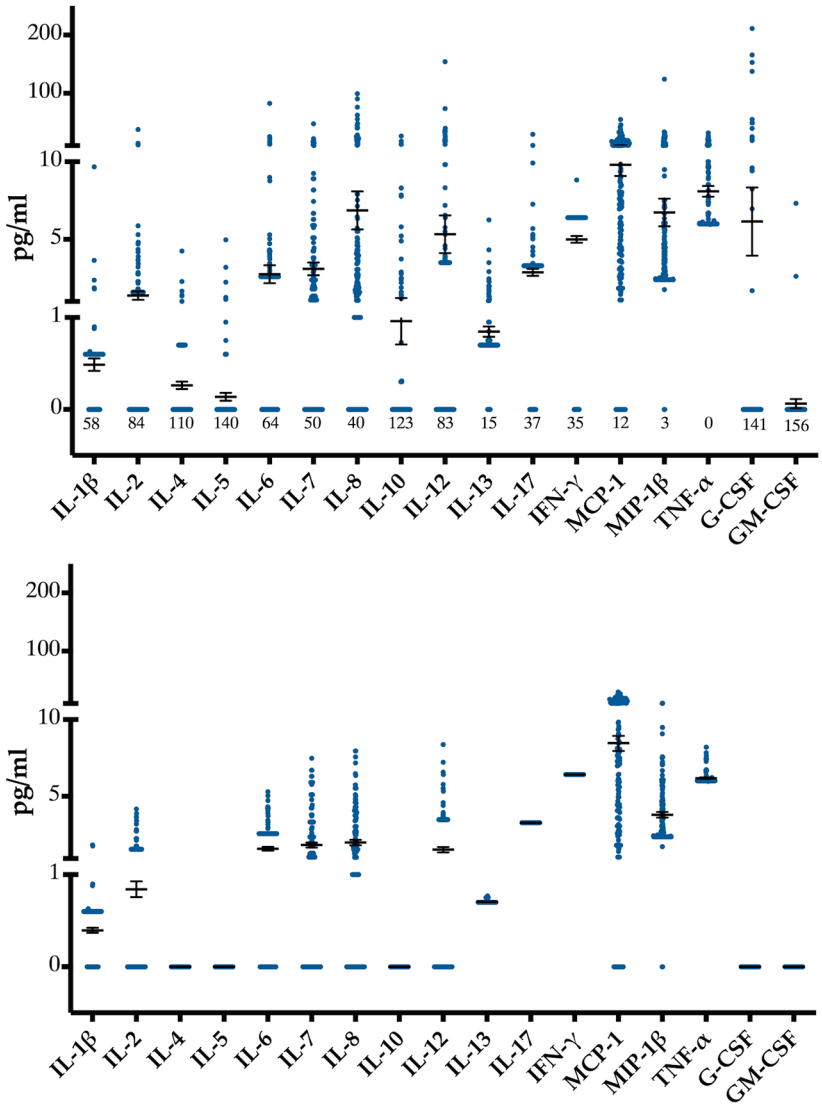

Figure A2. Scatter dot plots; the first is the distribution of the raw data, the second is the distribution of the clean data after the ROUT test.

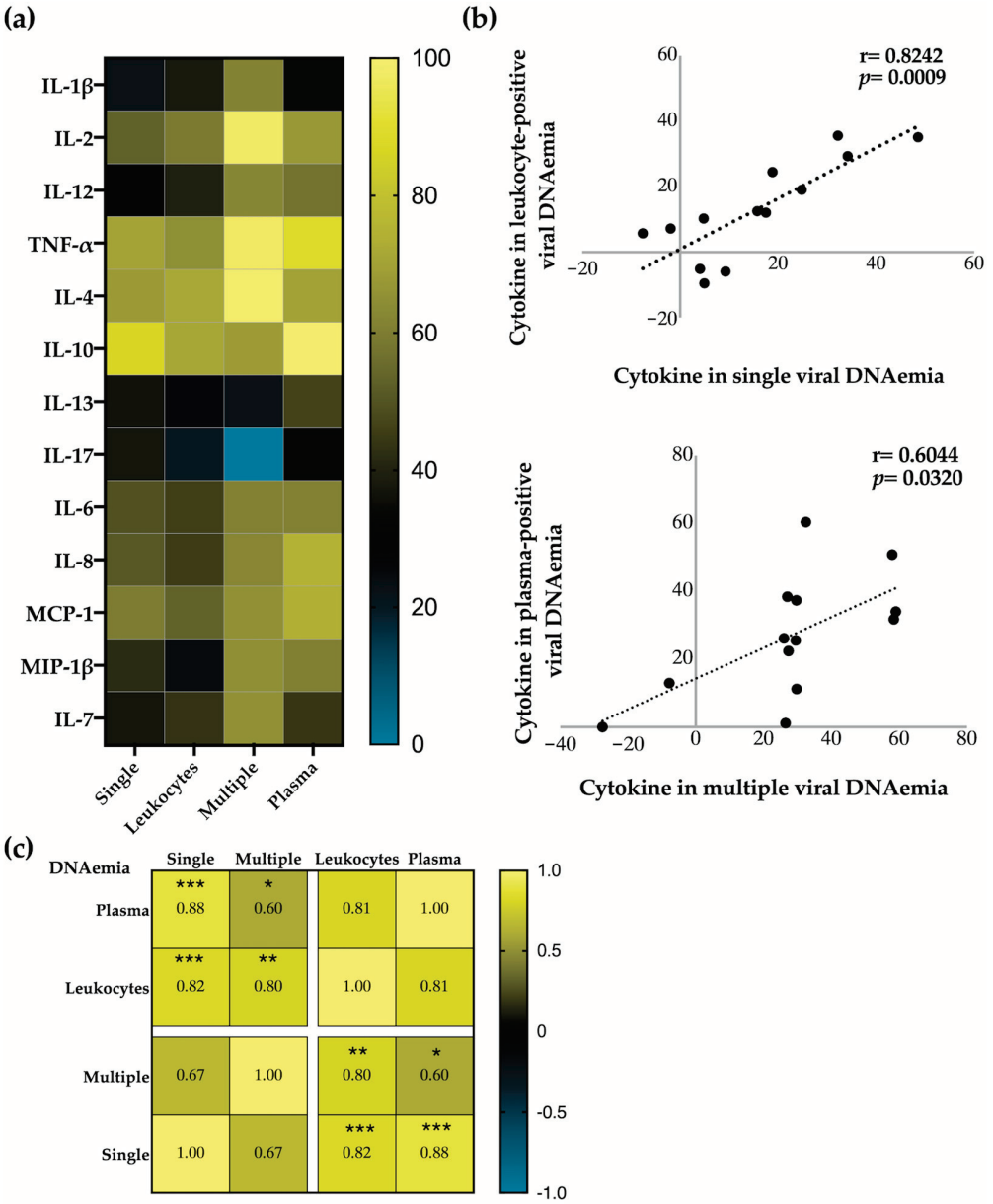

Figure A3. Correlation analysis of HCMV detection and cytokine concentration. (**a**) Heat map representing the comparison of cytokines between samples without viral DNAemia vs. four groups: column (1) HCMV positive samples in single detection; (2) HCMV positive samples detected in the leukocyte fraction, (3) HCMV positive samples in co-detection with other herpesvirus, and (4) HCMV positive samples detected in the plasma fraction. The increase in cytokines is shown in yellow and the decrease in blue. (**b**) Linear regression plots showing the correlation between cytokines levels present in samples with single viral DNAemia versus viral DNAemia detected in leukocytes (**upper panel**) and cytokines in multiple viral DNAemia vs. viral DNAemia in plasma (**lower panel**). (**c**) Spearman correlation matrix showing the correlative indexes between the four groups analyzed. Significant values * $p < 0.1$, ** $p < 0.01$, and *** $p < 0.001$.

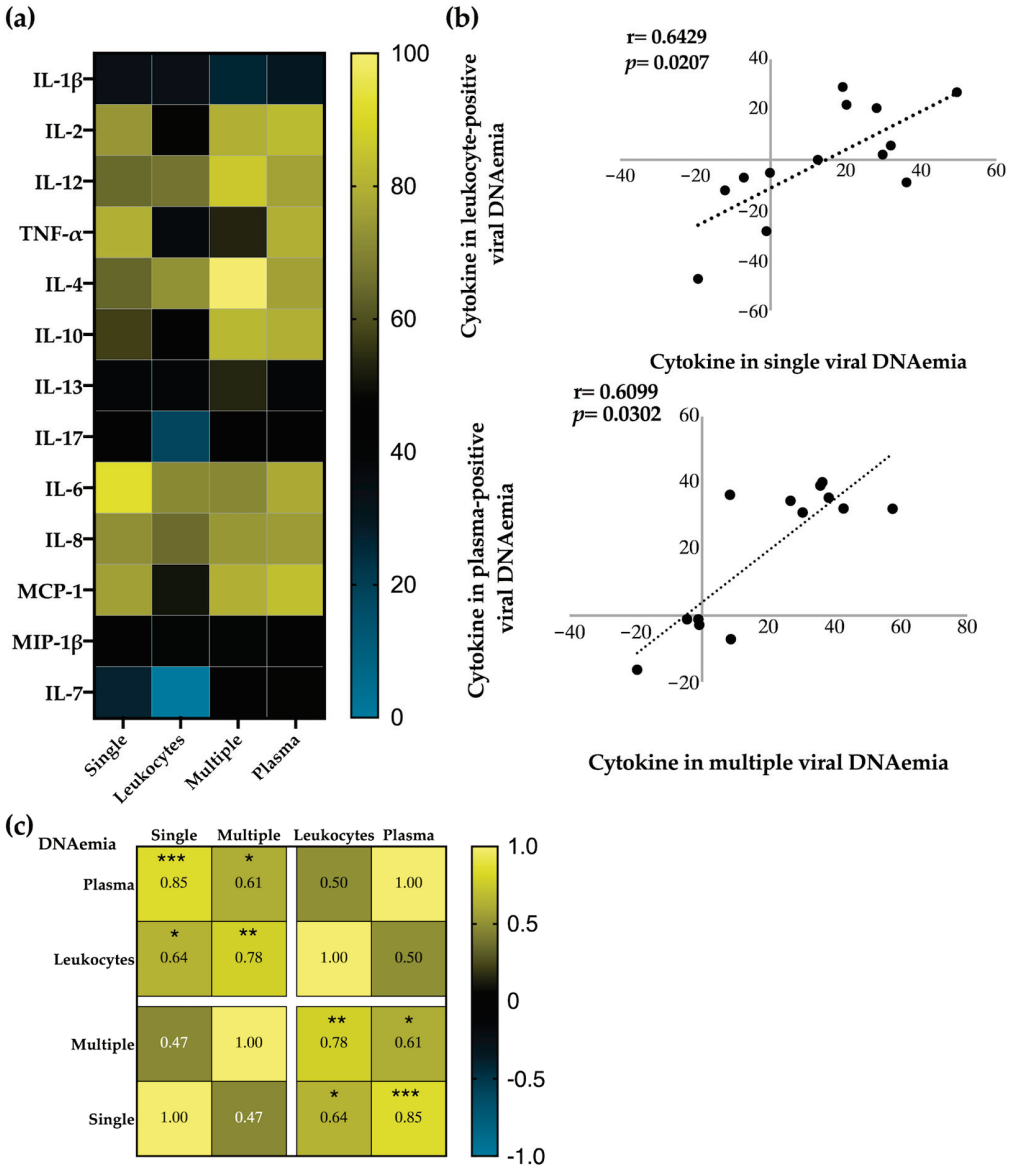

Figure A4. Correlation analysis of HHV6 detection and cytokine concentration. (**a**) Heat map representing the comparison of cytokines between samples without viral DNAemia vs. four groups: column (1) HHV6 positive samples in single detection; (2) HHV6 positive samples detected in the leukocyte fraction, (3) HHV6 positive samples in co-detection with other herpesvirus, and (4) HHV6 positive samples detected in the plasma fraction. The increase in cytokines is shown in yellow and the decrease in blue. (**b**) Linear regression plots showing the correlation between cytokines levels present in samples with single viral DNAemia versus viral DNAemia detected in leukocytes (**upper panel**) and cytokines in multiple viral DNAemia vs. viral DNAemia in plasma (**lower panel**). (**c**) Spearman correlation matrix showing the correlative indexes between the four groups analyzed. Significant values * $p < 0.1$, ** $p < 0.01$, and *** $p < 0.001$.

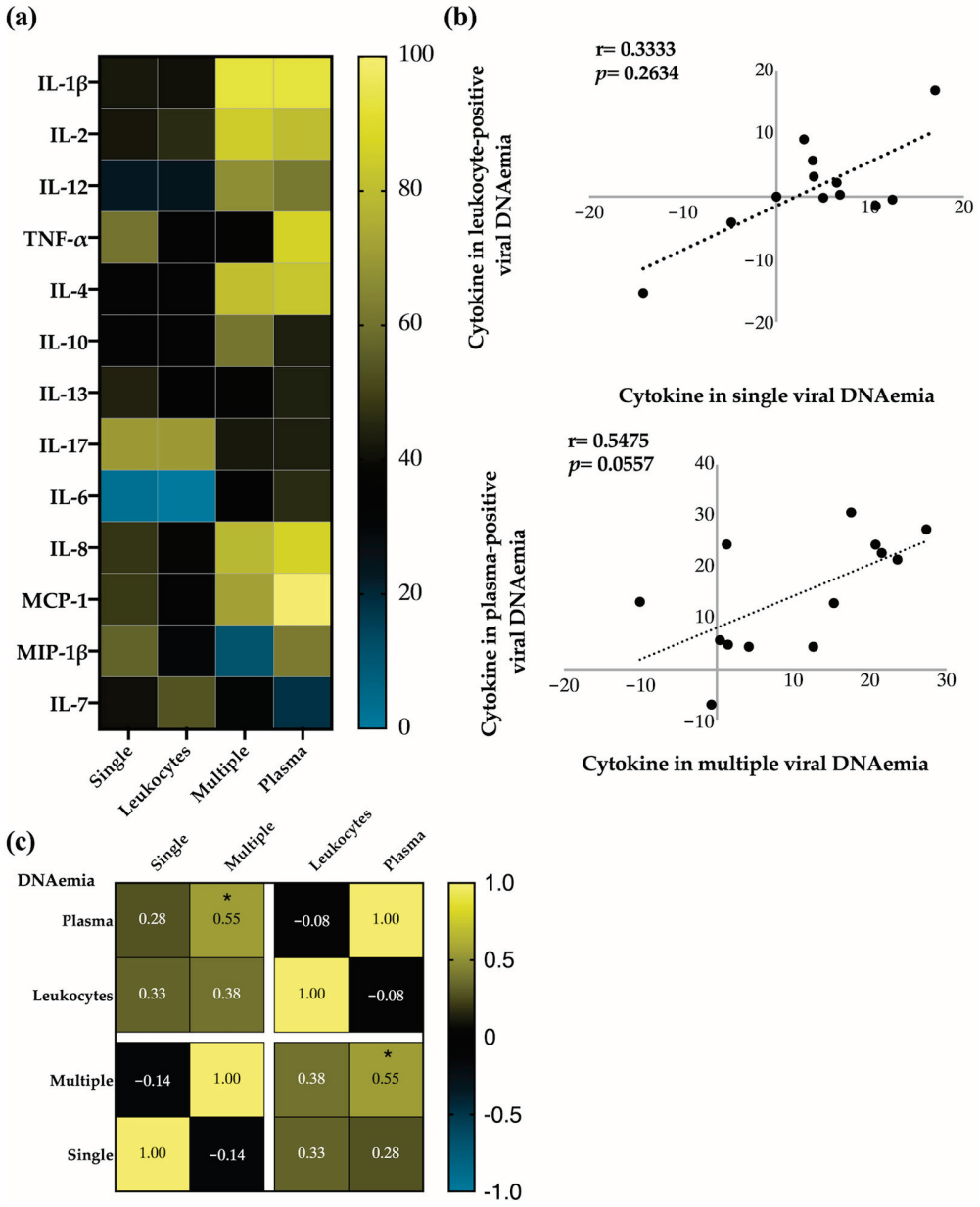

Figure A5. Correlation analysis of HHV7 detection and cytokine concentration. (**a**) Heat map representing the comparison of cytokines between samples without viral DNAemia vs. four groups: column (1) HHV7 positive samples in single detection; (2) HHV7 positive samples detected in the leukocyte fraction, (3) HHV7 positive samples in co-detection with other herpesvirus, and (4) HHV7 positive samples detected in the plasma fraction. The increase in cytokines is shown in yellow and the decrease in blue. (**b**) Linear regression plots showing the correlation between cytokines levels present in samples with single viral DNAemia versus viral DNAemia detected in leukocytes (**upper panel**) and cytokines in multiple viral DNAemia vs. viral DNAemia in plasma (**lower panel**). (**c**) Spearman correlation matrix showing the correlative indexes between the four groups analyzed. Significant values * $p < 0.1$.

Appendix B

The calculation of RR is based on a ratio of proportions, as outlined by the following equation:

$$\mathrm{RR} = \frac{a/(a+b)}{c/(c+d)}$$

where

- a represents the number of samples positive for both the cytokine and the outcome of interest (e.g., rejection or positivity to viral DNAemia);
- b is the count of samples positive for the cytokine but negative for the outcome;
- c indicates the samples negative for the cytokine but positive for the outcome; and
- d includes the samples negative for both the cytokine and the outcome.

This calculation method allows us to assess the risk of experiencing the outcome (e.g., rejection) when the exposure (cytokine) is present compared to when it is absent. Applying this analysis both to the association between cytokines and rejection, and between cytokines and each of the herpesviruses, enabled us to identify significant correlations that may influence transplant outcomes.

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Review

Cytomegalovirus Colitis in Adult Patients with Inflammatory Bowel Disease

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Abstract: Cytomegalovirus (CMV) colitis, a complication in patients with inflammatory bowel disease (IBD), particularly ulcerative colitis (UC), is a significant diagnostic and therapeutic challenge due to its overlap with IBD flares. CMV reactivation in IBD is driven by chronic inflammation, compromised immune function, and use of immunosuppressive agents like corticosteroids. Risk factors include older age, pancolitis, and severe disease. Diagnosis hinges on endoscopy and histology, with tissue biopsy and immunohistochemistry as the gold standard. Quantitative tissue PCR may aid in differentiating latent from active infection. CMV colitis exacerbates IBD symptoms, prolongs hospitalization, and increases colectomy rates. Antiviral therapy, primarily ganciclovir, improves outcomes in patients with corticosteroid-refractory UC. Treatment focuses on tapering corticosteroids, optimizing biologic therapies such as infliximab, and a careful application of antivirals tailored to disease severity and viral load. Further research is needed to refine diagnostic thresholds and treatment strategies to mitigate CMV's impact on IBD prognosis. Early identification and individualized management are critical to improving clinical outcomes and reducing morbidity.

Keywords: cytomegalovirus; inflammatory bowel disease; colitis

1. Introduction

Cytomegalovirus (CMV) is a ubiquitous double-stranded DNA virus belonging to the β-herpesviridae subgroup of the Herpesviridae family. Human CMV infection is very common, and most humans become infected during childhood. In the United States, CMV seroprevalence among adults is estimated to be 50%; however, this rate varies depending on age, geography, and socioeconomic status [1]. After CMV infection, CMV establishes a lifelong latent infection in CD34+ hematopoietic progenitor cells, CD14+ monocytes, macrophages, and dendritic cells. CMV infection can exist in a latent state, characterized by the presence of CMV viral DNA without active replication, or in an active state, marked by detectable viral replication, often without accompanying symptoms. During latency, CMV maintains a quiescent state with minimal viral gene expression, which helps it evade the host immune surveillance [2]. Primary CMV infection is often asymptomatic or can present as a mononucleosis-like syndrome in immunocompetent hosts. In contrast, CMV disease refers to the presence of clinical symptoms directly associated with active CMV infection, particularly in immunocompromised hosts. Clinical syndromes that may be observed in this setting include encephalitis, pneumonitis, hepatitis, uveitis, retinitis, colitis, and graft rejection [3,4].

CMV infection is more prevalent in patients with inflammatory bowel disease (IBD) than in the general population, with several factors increasing their susceptibility to latent

CMV reactivation. These include ongoing inflammation in the colon, poor nutritional status, compromised natural killer cell functionality, and the use of long-term immunosuppressive therapy for maintenance treatment [5]. Furthermore, the development of CMV colitis poses significant challenges in the management of IBD, particularly ulcerative colitis (UC). It usually presents with a combination of gastrointestinal and systemic symptoms that mimic an IBD flare, including bloody diarrhea, abdominal pain, fever, and weight loss. In some cases, severe complications such as toxic megacolon, colonic perforation, and death can occur. Additionally, CMV colitis can exacerbate UC symptoms, complicating the clinical picture and treatment course, particularly in patients with steroid-refractory UC, where symptoms persist despite immunosuppressive therapy. Other CMV end-organ damage is not commonly seen in IBD [6]. Here, we aim to review the latest evidence on the pathophysiology, diagnosis, and management of CMV infection in patients with IBD.

2. Risk Factors Contributing to CMV Colitis

Several risk factors and clinical indicators for CMV colitis in IBD patients have been identified, including demographics, patient-specific factors, and laboratory findings.

2.1. Demographic Factors

Females and older individuals have been shown to be more susceptible to CMV infection, with age consistently emerging as a risk factor for CMV reactivation [7–9]. This is further supported by a recent meta-analysis, which found that UC patients with a later age of disease onset are more likely to experience CMV reactivation [10].

2.2. Disease-Specific Factors

Disease severity and extent are other key risk factors for CMV colitis with pancolitis nearly doubling the risk of CMV infection compared to those with lesions confined to the left colon [11]. Similarly, a meta-analysis by Qin et al. found that the risk of CMV reactivation in severe UC was 1.5 times higher than in mild-to-moderate cases, and Lee et al. reported a 1.5-fold increased risk of CMV infection for each point increase in the Mayo score in patients with acute severe colitis [10,12].

2.3. Patient-Specific Factors and Laboratory Findings

Patient-specific risk factors include exposure to antibiotics, hypoalbuminemia, and elevated creatinine levels [8,13]. Clinically, CMV colitis is often linked to anemia, widespread colonic involvement during colonoscopy, and the presence of ulcers observed during endoscopy [9]. Additionally, reduced hemoglobin and elevated C-reactive protein levels are key laboratory predictors of CMV colitis [14].

2.4. Use of Immunosuppressants

The use of immunosuppressants also has a significant role in CMV reactivation and disease. Corticosteroids have been shown to increase the risk of CMV reactivation by fourfold. Lee et al. [6] reported that an average daily glucocorticoid dose exceeding 40 mg within one month significantly raises the likelihood of CMV reactivation. Furthermore, other studies have identified a cumulative glucocorticoid dose of more than 400 mg over a four-week period as a critical risk factor. Interestingly, 5-ASA use has been linked with a lower risk of CMV reactivation, possibly because treated patients have a milder disease and a subsequently lower incidence of CMV reactivation. A comprehensive analysis examining 2099 individuals revealed a notable link between CMV reactivation in UC patients and factors such as pancolitis, advanced age, and the use of immunosuppressants, azathioprine, and steroids [10,12].

Table 1 shows risk factors for CMV colitis in patients with IBD.

Table 1. Key risk factors for cytomegalovirus (CMV) colitis in patients with inflammatory bowel disease (IBD).

Category	Risk Factors
Demographic factors	• Older age • Female sex
Disease severity	• Severe UC • Pancolitis • High Mayo score
Clinical features	• Persistent anemia • Hypoalbuminemia • Elevated creatinine • Elevated CRP levels
Immunosuppressants	• Corticosteroid use (>40 mg/day or cumulative dose > 400 mg in 4 weeks) • Long term immunosuppressant use • Azathioprine or Thiopurine therapy
Colonoscopy findings	• Widespread colonic ulcerations
Other risk factors	• Antibiotic exposure • Poor nutritional status

3. Pathophysiology

IBD mainly encompasses two primary conditions: Crohn's disease (CD) and ulcerative colitis (UC). Anatomically, CD and UC affect the digestive tract differently. In UC, the rectum is consistently involved, along with continuous lesions in varying extents of the colon, whereas in CD, the lesions are patchy and discontinuous, occurring throughout the entire digestive tract. They are clinically characterized by alternating acute inflammatory flares and asymptomatic phases during remission [15]. In these patients, CMV seroprevalence is not well described but is assumed to be comparable to 50% of the general population in the United States [1]. CMV-seropositive individuals carry the latent CMV genome in various tissues, including the entire digestive tract, with the colon being a significant site for both viral latency and reactivation [16]. In the setting of chronic inflammation due to IBD, studies have shown that pro-inflammatory cytokines like tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) play a significant role in promoting CMV replication in seropositive patients. TNF-α, in particular, can stimulate CMV reactivation by activating the immediate early genes of the virus, which are critical for viral replication. Additionally, IL-6 contributes to CMV-related inflammation and further compromises cellular barriers, leading to viral spread and sustained inflammation in affected tissues, particularly in the gastrointestinal tract. These interactions create a vicious cycle of infection and inflammation [17,18]. However, it is worth noticing that the immune response in IBD varies between CD and UC. UC is associated with a TH2 cytokine profile, which does not effectively prevent CMV replication, whereas CD is associated with a TH1/TH17 profile that can inhibit CMV replication through the production of interferon-gamma (IFN-γ) [19]. This difference in immune response partially explains why CMV reactivation is more common in UC than in CD.

CMV colitis tends to develop in inflamed or ulcerated areas, triggered by mucosal damage and local inflammation. Furthermore, IBD patients receiving immunosuppressive therapy are exposed to both inflammation and weakened immune defense, two key factors that promote CMV reactivation [16]. Corticosteroids, which are commonly used as

the first line of treatment for moderate to severe IBD flare-ups, have been shown to promote CMV reactivation by downregulating the activity of monocytes and T-lymphocytes, which are critical in controlling latent viral infections. Additionally, corticosteroids facilitate the transcription of CMV's immediate early genes, promoting viral reactivation in infected cells [20,21]. Thiopurines promote apoptosis in T-lymphocytes by altering intercellular signaling pathways and have been shown to impair the function of CMV-specific T-lymphocytes and natural killer cells [22]. A recent meta-analysis involving 16 observational studies found that corticosteroid exposure in IBD patients doubled the risk of CMV reactivation in tissues, with an odds ratio (OR) of 2.10 and a 95% confidence interval (CI) of 1.31–3.37. In the same study, patients with UC treated with thiopurines also saw an increase in the risk of CMV infection [23].

4. Clinical Features

The clinical presentation of CMV infection in IBD patients can be diverse and often overlaps with symptoms of an IBD flare-up, making diagnosis particularly challenging [24]. Common manifestations include persistent or worsening abdominal pain, diarrhea, and rectal bleeding, which may be accompanied by systemic symptoms such as fever, weight loss, and fatigue. These symptoms can be indistinguishable from those of an IBD exacerbation, leading to potential delays in diagnosis and appropriate treatment. In severe cases, patients may develop life-threatening complications such as toxic megacolon or intestinal perforation, which require immediate medical intervention [25]. In addition, CMV infection can exacerbate the underlying IBD, leading to increased disease severity and resistance to conventional IBD treatments. This synergistic effect between CMV and IBD can result in a more aggressive disease course, prolonged hospitalization, and increased morbidity [26].

Endoscopic findings in CMV-infected IBD patients may reveal extensive ulcerations, with characteristic colonoscopic features of CMV-associated colitis including deep ulcers, punched-out ulcers, geographical ulcers, longitudinal ulcers, and mucosal defects [11]. Univariate logistic regression analysis revealed that severe easy bleeding was seen more frequently in CMV-positive patients than in CMV-negative patients (OR = 2.20, 95% CI: 1.14–4.28). Wide mucosal defects (OR = 4.58, 95% CI: 2.21–10.73), punched-out ulcerations (OR = 3.39, 95% CI: 1.78–7.46), longitudinal ulcerations (OR = 3.09, 95% CI: 1.66–6.26), and a cobblestone-like appearance (OR = 2.05, 95% CI: 1.11–3.82) were more frequently observed in CMV-positive patients than in CMV-negative patients [27].

The impact of CMV infection on long-term outcomes in IBD remains an area of active research, with evidence suggesting that CMV infection significantly correlates with poorer outcomes, such as prolonged hospitalization, colectomy, and increased mortality [12,28,29]. A meta-analysis further emphasizes the strong association between CMV infection and adverse IBD prognosis. Given its potential to complicate management and worsen outcomes, a high index of suspicion for CMV infection is essential in IBD patients with refractory or worsening symptoms as early recognition and appropriate treatment of CMV infection in this population can improve clinical outcomes and help prevent complications [30].

5. Diagnosis

A prompt and accurate diagnosis of CMV reactivation is crucial, especially in high-risk IBD patients, as it is a potentially reversible condition linked to poor clinical outcomes, particularly in corticosteroid-refractory UC [31]. Differentiating between an acute UC flare and CMV colitis can be challenging, as both conditions present with similar clinical symptoms, including fever, malaise, diarrhea, hematochezia, abdominal pain, and weight loss. While endoscopic findings like punched-out ulcers may suggest CMV colitis, no endoscopic feature is definitively pathognomonic for distinguishing between the two conditions [32,33].

Another critical distinction is between CMV infection and CMV disease. CMV infection, which may be detected through CMV serology, serum antigenemia, or positive polymerase chain reaction (PCR), does not always translate into active CMV disease. Active CMV disease is defined by the presence of symptoms or CMV-related tissue damage, and studies have shown that these noninvasive tests often correlate poorly with active disease [34,35].

The diagnosis of CMV colitis generally requires a combination of clinical evaluation, diagnostic testing, and a high index of suspicion for accurate identification. Endoscopic examination and histological analysis are crucial, and immunohistochemistry (IHC), along with tissue PCR, are key to confirming active CMV colitis in IBD patients and should be regarded as gold standard diagnostic tests [6]. Endoscopic findings suggestive of CMV colitis include ulcerations, erosions, and mucosal inflammation (Figure 1). Histological examination may reveal characteristic "owl's eye" inclusion bodies, which are pathognomonic for CMV infection (Figure 2). Left-colon biopsies identify most UC patients with CMV. Conversely, in CD, many patients had CMV detectable only in right-colon biopsies. A minimum of 11 biopsies for UC and 16 biopsies for CD was proposed in a study by McCurdy et al. to achieve an 80% probability of CMV detection [33]. The clinical significance of a positive PCR result for CMV DNA in colonic tissue without accompanying histological signs of infection is not well defined. The detection of viral DNA in the absence of histological evidence of infection is often interpreted as a sign of low-level reactivation or latent CMV infection. Consequently, it has been recommended that quantitative rather than qualitative PCR should be used, as higher viral loads are more closely associated with active CMV colitis and may predict better response to antiviral therapy.

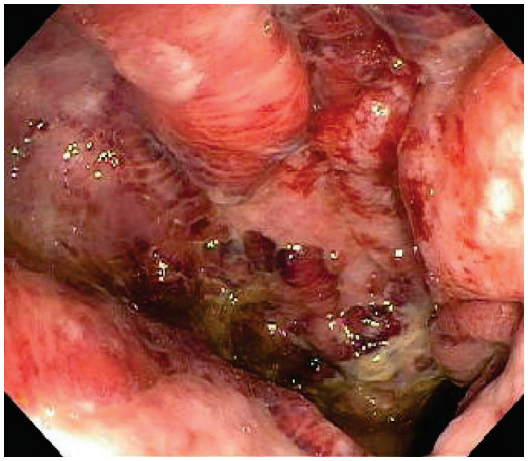

Figure 1. Colonoscopy image showing mucosal ulceration of the distal rectum in CMV colitis in an immunocompetent patient [36].

Viral culture, once considered the gold standard for CMV detection, is now obsolete in routine clinical practice due to limited sensitivity and its time-consuming nature. This method involves isolating the virus from tissue samples and growing it in cell culture. While it offers high specificity, the sensitivity of viral culture can be lower compared to more modern techniques like PCR. The main advantage of viral culture is its ability to detect viable, replication-competent viruses, which directly indicates active infection. However, the process typically takes 1–3 weeks to yield results, which may delay diagnosis and treatment initiation. Its clinical utility today is minimal, and CMV culture is no longer commercially available [3,38]. Furthermore, while viral culture was historically used to assess antiviral resistance through phenotypic testing, this is no longer a standard approach, as current practice favors genotypic testing [39].

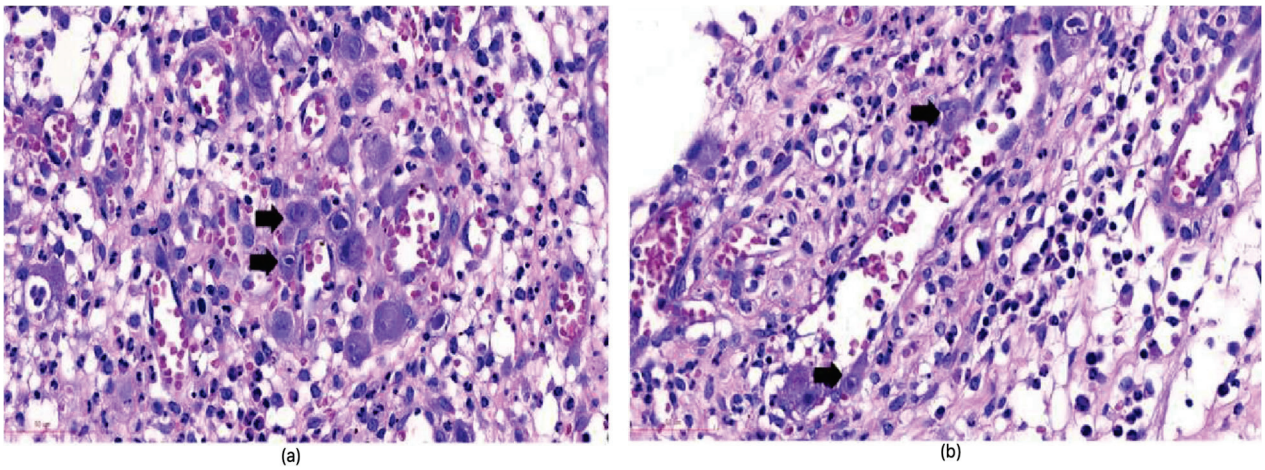

Figure 2. CMV-infected (**a**) mesenchymal cells from colonic tissue; (**b**) endothelial cells from colonic tissue. These CMV-infected cells have large ovoid nuclei with basophilic intranuclear inclusions (Cowdry bodies) surrounded by a clear halo (arrows) [37].

Noninvasive diagnostics, such as whole-blood PCR and pp65 antigenemia, are frequently used to assess systemic CMV viremia, especially in immunocompromised patients like transplant recipients, where they help guide pre-emptive antiviral therapy. However, their utility in predicting CMV colitis in IBD patients remains uncertain, as blood-based PCR detection does not always correlate with tissue-invasive disease in the colon. Studies have shown that while these tests are highly specific, they lack the sensitivity required to reliably detect colonic CMV reactivation in IBD patients [32,33]. Blood-based tests, including PCR, have a high positive predictive value (PPV), which can make a positive result useful in reducing the need for invasive endoscopic procedures. However, transient CMV viremia, which may not require treatment, complicates the interpretation of these tests. It remains unclear whether blood-based tests can accurately predict the viral burden in colonic mucosa, which is crucial for understanding disease severity and response to antiviral therapy [35]. Given these limitations, tissue biopsy remains the gold standard for diagnosing CMV colitis, particularly in patients with moderate-to-severe IBD, where early identification and treatment can significantly influence outcomes (Figure 3). Blood-based tests can serve as a valuable adjunct in determining when to withhold immunosuppressive therapy or in predicting the risk of colectomy, but they should not replace tissue-based diagnostics [34]. A summary of the investigations and of the diagnostic approach required for diagnosing CMV colitis is provided in Table 2 and Figure 4.

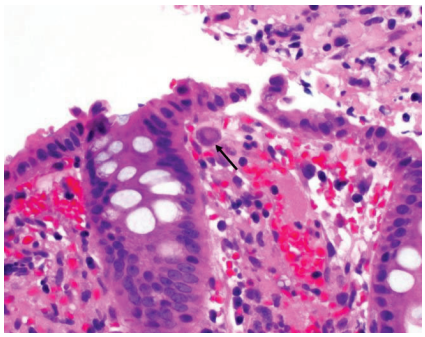

Figure 3. H&E stain of rectal biopsy, showing a large nucleus (arrow) with a smudged, eosinophilic chromatin pattern consistent with cytomegalovirus colitis [36].

Table 2. Investigations for the diagnosis of CMV colitis in patients with IBD.

Investigation	Findings	Comments
Endoscopy	Punched-out ulcers, erosions, mucosal inflammation	Unable to distinguish between CMV colitis and IBD flare
Histology	Owl's eye inclusions	Pathognomonic, gold standard
Immunohistochemistry (IHC)	CMV antigens in tissues	Sensitive and specific, gold standard
Tissue PCR	CMV DNA in tissues	Quantitative PCR preferred, high viral load correlated with active disease
Whole-blood PCR and pp65 antigenemia	CMV DNA and pp65 antigen in blood	Assesses systemic viremia, poor correlation with CMV colitis
Viral culture	Grows viable virus	High specificity, low sensitivity, slow turnaround time

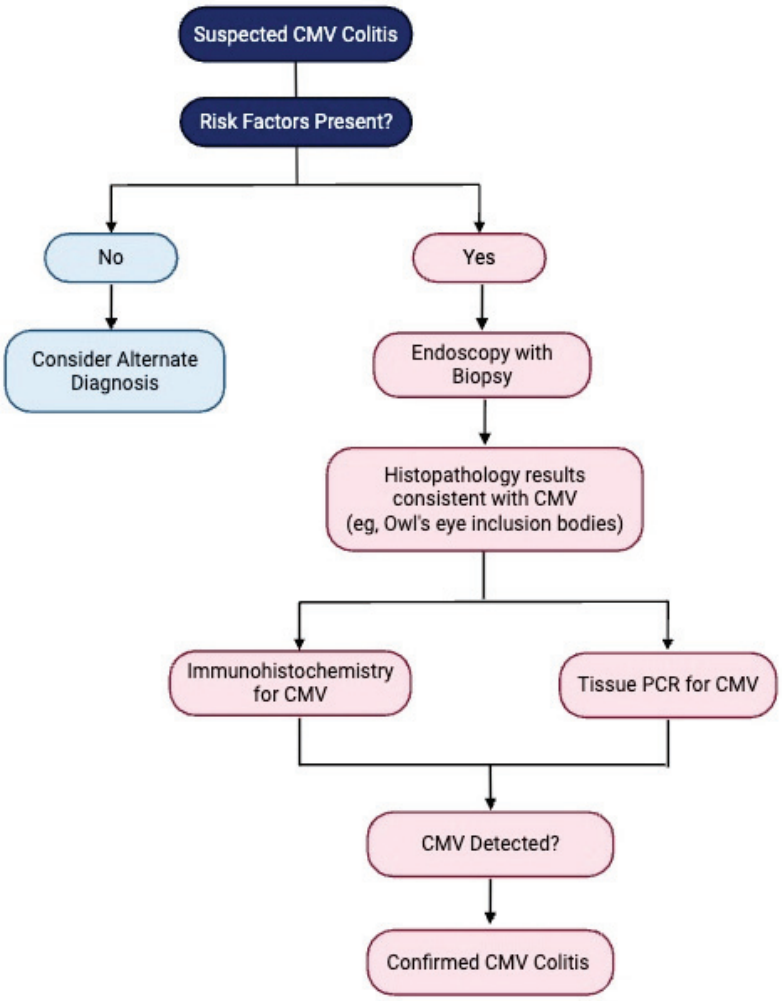

Figure 4. Diagnostic approach for CMV colitis.

6. Treatment Strategies for CMV in UC Patients

The treatment of CMV infections in IBD patients depends on the severity of the infection and the patient's immunosuppressive status. CMV can cause IBD exacerbations, especially in patients with corticosteroid-refractory UC [31]. CMV DNAemia is not unusual

in IBD patients receiving immunosuppression, and low-level reactivation may disappear without antiviral treatment [40]. Conversely, when CMV is found in the colonic tissue of high-risk patients, it is linked to a higher risk of colectomy, death, and increased healthcare use, suggesting it may play a role in worsening the disease [41,42]. In cases of high viral load or severe inflammation, including deep colonic ulcers, antiviral treatment has been shown to improve outcomes in patients who have not responded to immunosuppressive therapies [43]. A meta-analysis found that antiviral therapy reduced the risk of colectomy by 80% in CMV-positive UC patients who were resistant to corticosteroids, emphasizing the importance of early diagnosis and timely antiviral treatment in high-risk cases [44].

6.1. Role of Immunosuppressants and Biologics

Upon diagnosing CMV colitis, the focus should shift to gradually tapering corticosteroids, and its use should be reconsidered only once CMV infection has been properly treated. There is limited direct evidence on the optimal timing for initiation of biological therapies in this setting. However, an alternative anti-inflammatory should be introduced to promote remission. Infliximab is generally preferred over ciclosporin when feasible, given the potential TNF-avidity of CMV [45]. In a case series involving 23 patients, Minami et al. reported association between ciclosporin treatment and increasing likelihood for CMV reactivation [46]. Conversely, monoclonal antibodies targeting TNF-alpha, such as infliximab and adalimumab, have not been found to increase the risk of CMV reactivation. Studies show that CMV tissue infection is not associated with clinical resistance to these therapies. The beneficial effects of anti-TNF-α therapies on CMV infection are likely due to the reduction in TNF-α pro-inflammatory actions, which can otherwise promote viral replication. As a result, these biotherapies are recommended for managing moderate to severe flare-ups of IBD, particularly in cases complicated by CMV colonic infection [10,16].

Additionally, thiopurines should be discontinued, at least temporarily [23]. Vedolizumab is a monoclonal therapeutic antibody that is an antagonist to α4β7 integrin which is expressed specifically by gastrointestinal-homing T lymphocytes. This unique gut selectivity by Vedolizumab offers a more beneficial patient safety profile as compared to other biologics that have action on multiple targets to reduce inflammation [47]. Vedolizumab is generally regarded as a safe biologic when co-administered with antivirals [48–50], with a case reporting development of CMV colitis while on Vedolizumab [51]. However, its potential systemic effects warrant caution, and its use should be carefully considered in individuals with CMV colitis.

Studies evaluating safety of Tofacitinib in IBD patients reported rare occurrences of CMV colitis [52,53]. Ustekinumab was linked to a favorable safety profile in a pooled safety analysis of phase 2/3 studies, with two cases of CMV colitis in UC patients, although they were receiving concomitant corticosteroids [54].

6.2. Antiviral Therapy

Guidelines differ on the timing of antiviral therapy initiation in IBD patients with CMV infection. Both the American College of Gastroenterology (ACG) and the European Crohn's and Colitis Organisation (ECCO) recommend starting antiviral treatment in cases of moderate-to-severe colitis when histology reveals a high density of CMV in mucosal tissue and in those who are corticosteroid-refractory or corticosteroid-dependent [55]. There are limited data on the correlation between the progression of UC and tissue viral load, as assessed by viral inclusions through IHC or CMV DNA copy numbers. Some studies have shown that a higher colonic viral load correlates with an increased risk of colectomy, suggesting the potential benefit of antiviral therapy for CMV reactivation in UC patients [41,56]. However, the precise threshold for determining which patients may benefit

from antiviral treatment remains undefined. Although conclusive data on the use, mode of administration, and duration of antiviral treatment in CMV colitis are lacking, the ECCO, based on extensive experience with stem cell and solid organ transplant recipients, suggests using intravenous ganciclovir at a standard dose of 5 mg/kg every 12 h. If patients respond within 3 to 5 days, they can be transitioned to oral valganciclovir, generally at a dose of 900 mg twice daily. Treatment duration is individualized based on clinical and virologic response, with a minimum course of 2 weeks, extendable to 6 weeks or longer if necessary. For patients with a detectable viral load, weekly blood PCR testing can help monitor and guide ongoing therapy until the DNAemia is cleared. In cases without measurable viral load, symptom improvement—particularly in diarrhea—serves as the primary indicator of treatment response. If symptoms persist, repeat endoscopic evaluation may be needed. Myelosuppression is the most common severe side effect which requires weekly blood counts. G-CSF support is uncommon but may be necessary. Dose adjustments are crucial for those with renal impairment to avoid overdosing, which can cause drug toxicity, as well as underdosing, which can lead to treatment failure and potential resistance. Foscarnet may be used for patients intolerant to ganciclovir or in rare cases of ganciclovir-resistant CMV. Strict monitoring of renal function and electrolytes is essential. Concomitant administration of normal saline can help reduce the risk of irreversible renal damage. High concentrations of the drug are excreted in the urine, which may cause significant irritation and ulceration in the genital area. Maintaining careful hygiene can mitigate this risk [6].

Maribavir, a viral U97 kinase inhibitor, has been FDA-approved for the treatment of refractory or resistant CMV infection [57]. However, there is lack of significant clinical experience with Maribavir in the setting of IBD-associated CMV colitis, and its role in this context remains to be defined. Figure 5 shows a schematic representation of treatment approach for CMV colitis.

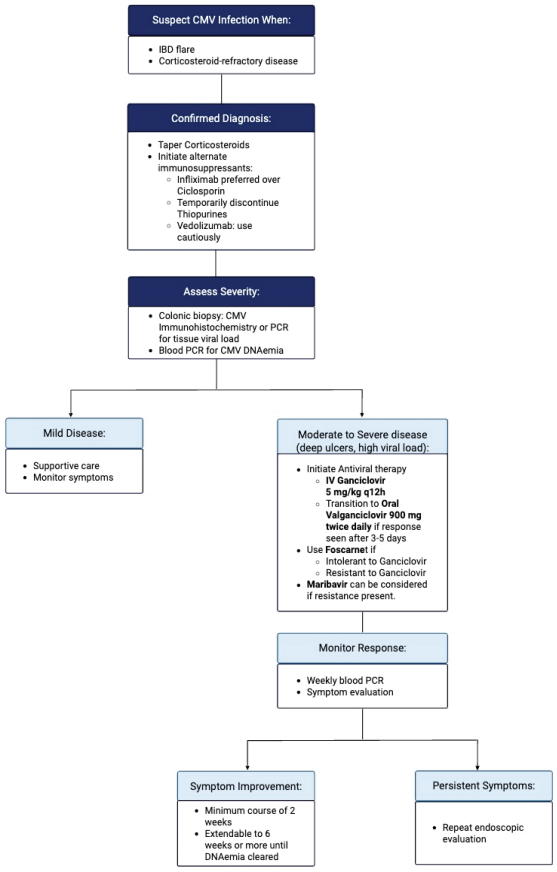

Figure 5. Treatment approach for CMV colitis.

6.3. Prophylaxis

While antiviral prophylaxis is used routinely in transplant recipients, there is little evidence to guide primary or secondary prophylaxis in patients with IBD; furthermore, the potential for adverse events does not justify standard chemoprophylaxis [58].

7. Prognosis and Negative Prognostic Factors

Patients' prognosis in CMV colitis is determined by multiple factors. CMV infection is negatively influenced by prolonged hospitalization, enhanced need for colectomy, and higher mortality rate [12,28,29]. In a study by Melotti et al., CMV DNAemia was shown to be a strong predictor of colonic CMV involvement and a negative prognostic marker, with the colectomy rate reaching 54.1% in DNAemia-positive patients compared to 34.4% in those without it [12].

Negative prognostic factors include the following:

- High colonic viral load (detected via immunohistochemistry or quantitative PCR).
- Extensive or deep ulcerations on endoscopy [11].
- Systemic inflammatory response—severe anemia, hypoalbuminemia, and elevated CRP levels [14].
- Older age and greater comorbidity burden, as measured by the Charlson Comorbidity Index [10,12].
- Corticosteroid refractory disease.

8. Conclusions

Cytomegalovirus (CMV) colitis represents a significant clinical challenge in patients with inflammatory bowel disease (IBD), particularly in those with ulcerative colitis (UC). Its overlapping presentation with IBD flares complicates a timely diagnosis and effective management. Accurate diagnosis relies on a combination of clinical evaluation and histological analysis, with tissue biopsy remaining the gold standard. Early identification of CMV infection in high-risk patients, particularly those with corticosteroid-refractory UC, allows for the initiation of targeted antiviral therapy, which has been shown to improve outcomes and reduce the need for colectomy. Treatment strategies should prioritize the tapering of corticosteroids, selective use of biologics, and the judicious application of antiviral agents such as ganciclovir or valganciclovir. Adopting individualized treatment plans based on disease severity, viral load, and patient response can optimize clinical outcomes while minimizing complications.

Further research is needed to refine diagnostic thresholds, define optimal antiviral regimens, and explore the role of biologic therapies in managing IBD complicated by CMV colitis. Clinicians must maintain a high index of suspicion for CMV colitis in patients with refractory or severe IBD symptoms to ensure timely and effective management, ultimately improving patient outcomes and quality of care.

Author Contributions: K.S. and A.P. reviewed the literature and drafted the manuscript. A.P. critically reviewed the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

ACG	American College of Gastroenterology
CI	Confidence Interval
CD	Crohn's Disease
CMV	Cytomegalovirus
ECCO	European Crohn's and Colitis Organisation
G-CSF	Granulocyte-Colony Stimulating Factor
IHC	Immunohistochemistry
IBD	Inflammatory Bowel Disease
IFN-γ	Interferon-gamma
IL-6	Interleukin-6
OR	Odds Ratio
PCR	Polymerase Chain Reaction
PPV	Positive Predictive Value
TNF-α	Tumor Necrosis Factor-alpha
UC	Ulcerative Colitis

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viruses

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Review

Treating Adenovirus Infection in Transplant Populations: Therapeutic Options Beyond Cidofovir?

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Abstract: Adenovirus (AdV) infections can lead to significant morbidity and increased mortality in immunocompromised populations such as hematopoietic stem cell and solid organ transplant recipients. This review evaluates currently available and emerging therapies for AdV infections. Cidofovir, while most commonly used, is limited by its variable efficacy and nephrotoxicity. This led to the development of brincidofovir, which has a better safety profile and great in vitro potency against AdV. The use of ribavirin and ganciclovir has been reported in the literature, but their use is limited due to inconsistent efficacy. Immune-based approaches, such as adoptive T-cell therapy, have shown promise in achieving viral clearance and improving survival but remain constrained by challenges related to manufacturing complexity and risks of graft-versus-host disease. This review underscores the need for standardized treatment protocols as well as comparative studies to identify optimal dosing and timing to initiate treatment. Future research should focus on individualized treatment approaches and the development of novel therapeutic agents to address the unmet clinical needs of AdV management.

Keywords: adenovirus; adoptive T-cell therapy; brincidofovir; cidofovir; ganciclovir; ribavirin; hematopoietic stem cell transplant; solid organ transplant

1. Introduction

Adenovirus (AdV) was first isolated from adenoidal tissues in 1953 by Wallace Rowe et al. while studying poliovirus and was termed an "adenoid degeneration agent" [1]. Shortly after, it was identified as the causative agent of an epidemic of acute respiratory illness among military service personnel [2]. Since then, this virus has been recognized as a human pathogen that can cause a myriad of clinical manifestations. While most AdV infections are mild and self-limiting, severe and fatal AdV infections began to emerge among leukemic and transplant patients in the 1970s [3] and spurred efforts to develop effective antiviral treatments for this virus.

Human AdV belongs to the family of *Adenoviridae*, which consists of viruses with non-enveloped, icosahedral virions containing linear dsDNA genomes of 25–48 kb and with the capability to infect various kinds of vertebrate hosts [4]. It is important to appreciate that human AdV is a family of viruses. As of March 2024, there are 116 "types" of human AdV identified [5], and this list will undoubtedly grow. AdV are classified into seven distinct species, from A to G, based on the guanine and cytosine content of the DNA genome. The initial 51 types were identified as "serotypes" through neutralization assays, while the remaining types were classified as "genotypes" based on bioinformatic analysis

of their whole-genome sequences [6,7] (Table 1). These viruses differ not only in their serotype-specific neutralizing epitopes and/or genomic size and sequences, but they also use different receptors for cell entry and different trafficking pathways within the host cell. These properties likely play important roles in their tropism and pathogenicity [8,9]. Species A, C, D, E, and F attach to the host cells via the CAR (Coxsackie virus and adenovirus) receptor, while group B species use CD46, which is a membrane cofactor protein [8]. Different species or serotypes/genotypes are well known to affect various host populations and produce different spectrums of clinical diseases (Table 2). Notably, their responses to antiviral therapy may also vary. However, efforts to evaluate the efficacy of antivirals against specific species, serotypes, or genotypes have been limited. In clinical settings, most laboratories diagnose AdV infections by amplifying and detecting highly conserved regions of the hexon gene, but such molecular diagnostic methods lack the capability to identify the specific species, serotype, or genotype of the virus.

Table 1. Classification of human adenovirus (hAdV) serotypes and genotypes.

Species		Human Serotypes and Genotypes
hAdV-A	*Mastadenovirus adami*	12, 18, 31, 61
hAdV-B	*Mastadenovirus blackbeardi*	**Subspecies B1**: 3, 7, 16, 21, 50, 64, 66, 68, 76, 114 B1/B2 recombinants: 77, 78 **Subspecies B2**: 11, 14, 34, 35, 55, 79, 106
hAdV-C	*Mastadenovirus caesari*	1, 2, 5, 6, 57, 89, 104, 108
hAdV-D	*Mastadenovirus dominans*	8, 9, 10, 13, 15, 17, 19, 20, 22–30, 32, 33, 36–39, 42, 49, 51, 53, 54, 56–60, 62, 63, 65, 67, 69, 70, 71–73, 74, 75, 80–88, 90–103, 105, 107, 109, 110, 111, 112, 113, 115, 116
hAdV-E	*Mastadenovirus exoticum*	4
hAdV-F	*Mastadenovirus faecale*	40, 41
hAdV-G	*Mastadenovirus russelli*	52

(Adapted from Kajon, A. E., 2024 [7], and personal communication.) Classification of hAdV serotypes and genotypes described to the present.
-- Types originally described as "serotypes" based on their distinct antigenic reactivities in neutralization assays. Currently also designated as genotypes 1–51.
-- and --Intertypic recombinant genotypes.
-- Intertypic recombinant genotypes with novel hexon genes.
52: Genotype of probable simian origin.

Table 2. Clinical diseases caused by adenovirus infection.

Clinical Disease	Populations at Risk	Causal Adenovirus types
Pharyngitis	Infants, children	1–7
Pharyngoconjunctival fever	Children	3, 7
Pertussis-like syndrome	Children	5
Pneumonia	Infants, children Military recruits	1–3, 21, 56 4, 7, 14
Acute respiratory disease	Military recruits	3, 4, 7, 14, 21, 55
Conjunctivitis	Children	1–4, 7
Epidemic keratoconjunctivitis	Adults, children	8, 11, 19, 37, 53, 54
Gastroenteritis	Infants Children	31, 40, 41 2, 3, 5
Intussusception	Children	1, 2, 4, 5
Hemorrhagic cystitis	Children HSCT, renal transplant recipients	7, 11, 21 34, 35
Meningoencephalitis	Children, immunocompromised hosts	2, 6, 7, 12, 32
Hepatitis	Pediatric liver transplant recipients	1–3, 5, 7
Nephritis	Renal transplant recipients	11, 34, 35
Myocarditis	Children	7, 21

Table 2. *Cont.*

Clinical Disease	Populations at Risk	Causal Adenovirus types
Urethritis	Adults	2, 19, 37
Disseminated disease	Neonates, immunocompromised hosts	1, 2, 5, 11, 31, 34, 35, 40

Adapted from Mandell, Douglas, and Bennett's *Principles and Practice of Infectious Diseases* (2020; 9th edition), Chapter 142 on adenoviruses [10]. Abbreviations: HSCT, hematopoietic stem cell transplant.

From multicenter studies conducted across the United States, Europe, and Japan, the incidence of AdV infection in adults undergoing hematopoietic stem cell transplantation (HSCT) ranges from 2.99% to 6%. This incidence is significantly higher in pediatric HSCT patients, ranging from 23% to 32% [11–13]. Similarly, in solid organ transplant (SOT) patients, the incidence of AdV infection is higher in pediatric populations compared to adults. The incidence also varies by organ type, with intestinal transplants showing a higher prevalence of infection compared to other organs [14] (Table 3).

Table 3. Incidence of adenovirus infection by organ transplanted.

Allograft Type	Reported Adenovirus Incidence
Pediatric Transplantation	
Liver	3.5–38%
Heart, heart–lung, lung	7–50%
Kidney	11%
Intestinal, multivisceral	4.3–57.1%
Adult Transplantation	
Liver	5.8%
Heart, heart–lung, lung	6–22.5%
Kidney	4.1–6.5%
Intestinal, multivisceral	NA

Adapted from Florescu et al., 2019 [14].

While most AdV infections are self-limiting, they can lead to fatal outcomes, particularly in neonates and immunocompromised hosts. In immunocompromised patients, AdV infections can manifest with a wide spectrum of clinical presentations, ranging from asymptomatic viral shedding to severe disseminated disease. Various organ systems may be affected, including the central nervous system (CNS), lungs, liver, gastrointestinal (GI) tract, kidneys, and bladder. Among HSCT patients, AdV infections are associated with high mortality rates, up to 80% in some studies [15–17]. Mortality rates of up to 53% are reported in pediatric liver transplant patients [18].

Despite its potential to cause severe or life-threatening disease, therapeutic options for AdV infections remain limited. Clinical outcomes are highly variable based on host factors, immune status, and organs involved. Additionally, these therapies are limited by frequent and significant toxicity, which often exacerbates pre-existing end-organ dysfunction resulting from both underlying patient factors and the AdV infection itself. Current guidelines from transplant societies recommend cidofovir (CDV) as the antiviral agent of choice for AdV treatment; however, data on other antiviral drugs, such as ribavirin (RBV) and ganciclovir (GCV), as well as newer treatment options like brincidofovir (BCV) and adoptive T-cell therapy, have not been systematically evaluated [14,19,20].

This review critically explores current and emerging therapeutic options, including both antiviral agents and immune-based therapies such as adoptive T-cell treatments. In particular, we will assess the available clinical data regarding the efficacy, adverse effects,

and clinical challenges associated with these anti-AdV modalities in the management of AdV infections among HSCT and SOT populations.

2. Cidofovir (CDV)

CDV [(S)-1-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine or (S)-HPMPC] is an acyclic nucleoside phosphonate with a wide spectrum of activity against DNA viruses. De Clercq et al. first described the antiviral activity of (S)-1-(3-hydroxy-2-phosphonylmethoxypropyl)adenine [(S)-HPMPA] in 1986 [21]. In the following year, they introduced its analogue (S)-HPMPC, synthesized through substituting the adenine moiety of (S)-HPMPA with cytosine, and demonstrated its potent activity against varicella zoster virus (VZV), cytomegalovirus (CMV), and AdV [22]. CDV in its phosphorylated form is a competitive inhibitor of DNA polymerase, and once incorporated into the viral DNA, it blocks further DNA synthesis, thereby interfering with viral replication [22].

CDV was primarily studied and subsequently employed clinically for severe CMV infections in AIDS patients in the 1990s. It was FDA approved in 1996 for CMV retinitis in patients with acquired immunodeficiency syndrome (AIDS), and this remains its sole approved indication at the time of writing. CDV has been employed off-label primarily as salvage therapy or in the setting of antiviral resistance in a variety of other viral infections, including herpes simplex virus (HSV), VZV, Epstein–Barr virus (EBV), BK polyomavirus, JC polyomavirus, and so on. It has also been used as a topical preparation for HSV and human papillomavirus (HPV), as well as via intravitreal and intravesicular injection for CMV and BK virus, respectively [23,24]. Also known for its activity against poxviruses, CDV has recently been investigated for the treatment of Mpox as well as smallpox [25,26].

The major treatment-limiting toxicity of CDV is nephrotoxicity, which is dose-dependent and may manifest as increased serum creatinine, proteinuria, azotemia, and Fanconi-like syndrome with glycosuria and hypophosphatemia [27–29]. The nephrotoxicity of CDV is due to its extensive transport by the basolateral anion transport system of the proximal renal tubular epithelium, resulting in severe necrosis and degeneration of proximal convoluted tubule cells [30,31]. Probenecid, as a potent inhibitor of this transport system, was found in animal models and subsequently in human studies to mitigate this toxicity while not affecting CDV's concentration in non-renal tissues [29,32,33]. Thus, a variety of toxicity-mitigating strategies, including pre-hydration, limiting dose frequency, and concomitant administration of probenecid, were employed in initial clinical trials and are still used in contemporary practice [28,29].

The in vitro activity of CDV against human AdV was first described by De Clercq et al. in 1987 in human embryonic lung cells. The minimum antiviral concentration was found to be 3.4 ug/mL, far below the minimum cytotoxic concentration, suggesting promise as a potentially viable agent against clinical human AdV infection [22]. Subsequent in vitro studies have found variable CDV IC_{50} values against human AdV but, for the most part, within clinically observable drug concentrations [34,35]. An attractive in vitro attribute of CDV is its broad activity against a wide variety of human AdV species (A through F) [36], but CDV-resistant variants could emerge by serial passaging [37].

The first use of CDV for the treatment of AdV infection was reported by Hedderwick et al. in 1998 [38]. The case involved a 40-year-old male with AIDS who developed AdV-related cholecystitis and colitis, confirmed by histopathology. The patient underwent a cholecystectomy, and his symptoms of diarrhea initially resolved with CDV administered at 5 mg/kg per week. However, the therapy was discontinued after two doses due to renal toxicity, and his diarrhea subsequently recurred. This was followed by a report of successful treatment of AdV colitis in a 17-year-old umbilical cord HSCT recipient. In this case, the patient was treated with CDV after an apparent failure of RBV therapy [39].

Subsequent to these single case reports, larger studies of AdV treatment in HSCT populations began to emerge. Legrand et al. [40] described a cohort of seven pediatric HSCT patients with AdV infection, including three with disseminated disease. Among them, CDV treatment was successful in five cases.

These early studies utilized a CDV dosing of 5 mg/kg/week intravenously (IV) for 2 weeks, then every other week (with concomitant probenecid), and associated renal toxicity of CDV was a major concern. A more "renal-protective" dosing of CDV at 1 mg/kg thrice weekly (TIW) was first reported in a prospective trial with pediatric HSCT patients [41]. Other reports using CDV at a dosage of 1 mg/kg TIW have been mostly limited to pediatric populations. Few studies [42,43] directly compared the efficacy and safety of the conventional dosing of 5 mg/kg/week versus the modified dosing of 1 mg/kg TIW. The two CDV regimens were overall well tolerated without any significant difference in nephrotoxicity, although Guerra Sanchez et al. [43] reported that the modified dosing had a higher, although non-significant, rate of viral load clearance and suggested that more frequent dosing at lower levels may be more efficacious. A recent pharmacologic review by Riggsbee et al. [44] analyzed 16 manuscripts with a total of 210 pediatric patients who received CDV for AdV. Of these, 63% of patients received the conventional dosing of 5 mg/kg/week and 37% of patients received the modified 1 mg/kg TIW dosing. Nephrotoxicity was reported in 18% of patients treated with the conventional regimen but only 4% of those on the modified regimen. A recent retrospective multicenter cohort study [45] examined the safety and efficacy of CDV in adult HSCT recipients. The study included 165 patients from nine centers who received CDV for AdV, CMV, or BK virus. Most patients (115; 69.7%) received CDV at 5 mg/kg/week, while the others received CDV at 1 mg/kg/week (18; 10.9%), 3 mg/kg/week (12; 7.3%), or 1 mg/kg TIW (11; 6.7%). Overall, 25% of these patients developed reversible nephrotoxicity, and for those who received CDV for AdV, 72% demonstrated virologic response. However, there were no comparisons made between the 5 mg/kg/week dosing and the lower doses. While it is reasonable to consider the modified dosing for adult patients with renal dysfunction, close monitoring of renal function and minimizing the use of other nephrotoxic drugs remain crucial. It is important to note that breakthrough infections with HSV and CMV have been reported with the 1 mg/kg TIW dosing [46,47] despite the fact that CDV has good activity against these herpesviruses [48].

In terms of the efficacy of CDV for AdV treatment, variable degrees of success have been reported in immunocompromised populations, ranging from 23% to 100% [41,42,49–52] for HSCT patients. Al-Heeti et al. [53] have recently reviewed studies of CDV for the treatment of AdV among SOT recipients. A majority reported successful outcomes, but this may be subject to publication bias. The discrepancies in CDV's effectiveness are likely multifactorial. Susceptibility to CDV might vary among different species, serotypes/genotypes, or isolates. Other adjunctive therapies, such as IVIG, might be used, although the utility of IVIG for AdV treatment is debatable. The severity of AdV infections might also differ, ranging from asymptomatic viremia or viral shedding to disseminated disease. Patient characteristics also varied, such as age (pediatric versus adult), type of transplant, and degree of immune recovery. In particular, the importance of lymphocyte reconstitution has been demonstrated in several studies [51,54,55]. As such, for very high-risk patients (e.g., haploidentical HSCT), the addition of donor lymphocyte infusion (see below) to CDV treatment may confer additional benefits [56].

An important consideration regarding AdV treatment with CDV is the timing to initiate therapy. According to a survey on the incidence and management of AdV infection after allogeneic HSCT conducted among European Bone Marrow Transplant (EBMT) centers, some initiated treatment with two consecutive PCR positivity irrespective of viral load (32/74 (43%)), or based on the viral load, most frequently at >1000 copies/mL (41%), fol-

lowed by 100–10,000 copies/mL (29%), >100 copies (21%), and >10,000 copies/mL (9%) [57]. In the USA, many centers use a threshold of >1000 copies/mL to initiate pre-emptive treatment in high-risk patients [13,58]. Currently, the guidelines from the European Conference for Infections in Leukemia (ECIL)-4 and the Infectious Disease Working Party (IDWP) of EBMT both recommend tapering immunosuppression as feasible and starting CDV for pre-emptive treatment in patients with AdV viral load >1000 copies/mL [19,59].

While CDV is currently considered a treatment of choice for AdV infections in immunocompromised patients, its utility is limited by renal toxicity and variable efficacy. For HSCT patients during the early post-transplant phase, early initiation of treatment to control viral replication and to allow time for immune recovery would likely be beneficial. Future studies should prioritize developing standardized treatment protocols and compare the effectiveness and side effects of dosing regimens. Additionally, tailoring treatment based on individual patient risks may further improve clinical outcomes.

3. Brincidofovir (BCV)

BCV (hexadecyloxypropyl-cidofovir; also known as CMX001) is a lipid conjugate form of CDV with a similar spectrum of activities against various viruses [60]. It was initially developed in response to the growing bioterrorism threat in the late 1990s and early 2000s [61]. In 2002, inspired by earlier successes in enhancing the oral bioavailability of acyclovir and ganciclovir through alkoxyalkyl esterification, Hostetler et al. applied a similar approach to cidofovir, synthesizing hexadecyloxypropyl-cidofovir—later named brincidofovir [62]. Studies in mouse models confirmed BCV's oral bioavailability and demonstrated reduced kidney accumulation, suggesting a lower risk of nephrotoxicity [63]. When compared with CDV, BCV also had dramatically increased antiviral potency owing to a unique cellular uptake and metabolism mechanism enabling >100-fold greater intracellular active drug levels and a prolonged intracellular half-life [64–66].

These findings have established several key pharmacokinetic and pharmacodynamic advantages of BCV, including enhanced antiviral efficacy, oral bioavailability, and reduced nephrotoxicity. BCV was FDA approved for the treatment of smallpox in 2021 and has been procured for the US Centers for Disease Control (CDC) Strategic National Stockpile for use against smallpox and other orthopoxviruses, including Mpox, as an investigational treatment [67].

BCV's activity against AdV was first demonstrated in 2005 by Hartline et al., who observed 5–200-fold greater antiviral potency against AdV serotypes 3, 5, 7, 8, and 31 in a human fibroblast model and then subsequently in animal models [68,69]. For clinical use, Florescu et al. [70] first reported the clinical experience of 13 immunocompromised patients who received oral BCV as emergency investigational new drug use (EIND) for treatment of AdV disease and viremia. Among these patients, 11 were allogeneic HSCT recipients, and the remaining two had severe combined immunodeficiency and small bowel transplant, respectively. In their report, 69% of patients exhibited a 99% reduction in viral load or complete resolution of viremia, with an overall survival rate of 77%. Notably, no serious adverse events were attributed to BCV, and there were no significant changes in renal function from baseline to week 8 of therapy. Subsequently, there have been a number of case reports/series as well as retrospective studies that describe the use of oral BCV for treatment of AdV infection in HSCT or SOT patients.

Among the case reports/series with 16 patients combined, 6 patients underwent SOT and 10 received allogeneic HSCT. A total of 12 were treated with CDV prior to receiving BCV. A total of 12 achieved infection resolution and survived, although 4 HSCT recipients died (Table 4). While these findings are promising, the validity of these reports may be affected by publication bias and the limited number of cases.

Table 4. Transplant patients with adenovirus infection treated with brincidofovir.

First Author [Reference]	Year of Publication	Number of Patients	Age/Sex	Underlying Condition	Onset of AdV from Tx	AdV Infection	Species/ Serotype	Other Treatment	Adjunctive Measure	Outcome	Comments
HCT Cases:											
Paolino [71]	2011	1	12 Y/F	Allo-HCT for aplastic anemia	D + 89	GI, liver, Lung	NA	CDV prior	IVIG, Failed CDV prior, Reduction in IS	Recovery	
Voigt [72]	2016	1	5 Y/F	Allo-HCT X 2 (MUD) for MDS	D + 237	Viremia	C	CDV prior	Failed CDV prior	Recovery	Also had resistant HSV-1 infection that resolved
Ramsay [73]	2017	3	59/F	MUD Allo for AML	D + 22	Lung, GI	NA	CDV prior	Reduction in IS	Recovery	
			57/F	MRD Allo for MM	D + 51	Lung, GI				Died, had viremia at time of death	
			34/M	Mismatched Allo for ALL	D + 21	GI, hepatic, urine			Reduction in IS	Recovery	
Meena [74]	2019	5	2.2 Y/M	MUD for MDS	D + 17	GI	NA	CDV prior		Recovery	Concomitant CMV and PIV infection
			11 Y/F	MUD for ALL	D + 18	GI, Lung		CDV prior		Died of sepsis	Concomitant CMV and rhinovirus infection
			10 Y/F	MRD for osteopetrosis	D + 38	GI, Lung		CDV prior		Recovery	Concomitant PIV, rhinovirus and RSV
			15 Y/F	Cord Blood transplant for AML	D + 303	GI		CDV prior	IVIG	Died of sepsis	Concomitant rhinovirus, sapovirus and EBV
			2.9 Y/M	MUD for CDA type 2	/	GI				Died of EBV pneumonitis	
SOT Cases:											
Sulejmani [75]	2018	2	44 F	Intestinal Tx	D + 30	GI	NA	Ribavirin and CDV prior	Reduction in IS	Recovery, 2 episodes of rejection	
			28 M	Intestinal Tx	6 years from Tx	GI		CDV prior	Reduction in IS	Recovery	
Londeree [76]	2020	4	17 M	Kidney Tx	D + 12	Bladder	NA	NA	Reduction in IS	Recovery	
			19 M	Kidney Tx	D + 912	Lung		CDV prior	Reduction in IS	Recovery	
			13 M	Liver-kidney Tx	D + 487	Bladder, kidney		CDV prior	IVIG, Reduction in IS	Recovery	
			9 mo F	Liver Tx	D + 33	Viremia, Liver		CDV prior		Recovery	

Table 4. *Cont.*

Clinical Studies (>10 patients)										
Author [Reference]	**Year**	**Type of study**	**No. of Patients**	**Median age (range)**	**Onset of AdV from Tx**	**Underlying condition**	**Treatment agents**	**Virologic response**	**Outcome**	**Adverse events/side effects**	**Comments**
Florescu [70]	2012	Retrospective Multi-center	13	6 (0.92–66)	D + 75 (15–720)	11 Allo HSCT, 1 SCID, 1 Intestinal Tx	All patients = CDV + BCV	A total of 9/13 patients (69.2%) achieved a VR * at week 8	The 8-week survival rate was 76.9%	None attributed to BCV	Compared with non-responders, complete responders had longer survival (median, 196 days versus 54.5 days; $p = 0.04$)
Ramsay [73]	2017	Retrospective Single-center	10	40 (17–66)	D + 65 (20–1140)	HSCT	2 = BCV 5 = CDV 1 = BCV + CDV	Complete VR in 2/3 who received BCV, 4/6 in CDV	Two patients survived and one died in BCV group. Four patients survived and two died in CDV group.	NR	
Hiwarkar [77]	2017	Retrospective Multi-center	41	5 (2 months to 18 years)		HSCT	18 = BCV 23 = CDV	Complete VR in 15/18 (83%) who received BCV and 2/23 9% in CDV	Thirty-nine patients survived and two patients who received CDV died from AdV infection	Severe abdominal cramps and diarrhea in 1 patient who received BCV	BCV led to major responses in 9 of 11 CDV-unresponsive patients
Thomas [78]	2021	Retrospective Single-center	93	4.07 (2.12, 10.5)	D + 60 (IQR 25–75%: 16–123 days)	HSCT	5 = BCV 13 = CDV 6= Both BCV + CDV 66= no Tx	A total of 4/5 in BCV and 5/6 in BCV + CDV had resolution of disease	A total of 47/93 (51%) patients died in the total cohort, no data reported separately about BCV or CDV	No statistically significant difference in the hazard of rise in creatinine, elevated LFTs or diarrhea between groups	
Perrucio [79]	2021	Retrospective Multi-center	30 patients with 44 episodes of AdV infection	10 (9 months–19 years)	D + 90 (20 days–14 months)	HSCT	23 = CDV 21 = BCV	In BCV group: CR 48% PR ** 9.5% Stable 9.5% Progressive 24% In CDV group: CR 36% PR 4% Stable 8% Progressive 54%	Overall survival was 30%, 13% mortality due to AdV	1 patient with GI toxicity related to BCV (5%)	The response rate was higher with BCV compared to CDV (67% vs. 47%, p = 0.05)

Table 4. *Cont.*

Clinical trial										
Author [Reference]	**Year**	**Type of study**	**No. of Patients**	**Median age (range)**	**Onset of AdV from Tx**	**Underlying condition**	**Treatment agents**	**Virologic response**	**Outcome**	**Adverse events/side effects**	**Comments**
Grimley [80]	2017	Randomized placebo controlled phase 2 trial for prevention of adenovirus disease in patients with adenoviremia	48	8 (0–55) in BCV 2 mg/kg BIW group 9 (2–70) in BCV 4 mg/kg QW group 11 (1–53) in placebo group	NA	HSCT	14 = BCV BIW 16 = BCV QW 18 = Placebo	67% in BCV BIW group 29% in BCV QW group 33% in placebo group	**Treatment failure:** 21% in BCV BIW group 38% in BCV QW group 33% in placebo group **All-cause mortality:** 14% in BCV BIW group 31% in BCV QW group 39% in placebo group	**Diarrhea** 57% in BCV BIW group 38% in BCV QW group 28% in placebo group **GI GVHD** 50% in BCV BIW group 25% in BCV QW group 17% in placebo group	No myelotoxicity or nephrotoxicity

* Virologic response (VR) was defined as achievement of ≥99% decrease in plasma viral load from baseline or undetectable viral load by the end of treatment or follow-up period. ** Partial response (PR) was defined as a >1 <2-log reduction in viral load by the end of treatment. **Abbreviations:** AdV, adenovirus; Allo-HCT, allogeneic hematopoietic cell transplant; ALL, acute lymphocytic leukemia; AML, acute myeloid leukemia; BCV, brincidofovir; BIW; twice weekly; CDV, cidofovir; CMV; cytomegalovirus; CR, complete remission; EBV, Epstein–Barr virus; GI, gastrointestinal; GVHD, graft-versus-host disease; HSV, herpes simplex virus; HSCT, hematopoietic stem cell transplant; IQR, interquartile range; IS, immunosuppression; IV, intravenous; IVIG, intravenous immunoglobulin; LFT, liver function tests; MDS, myelodysplastic syndrome; MM, multiple myeloma; mo, months; MRD, matched related donor; MUD, matched unrelated donor; NA, not available; PIV, parainfluenza virus; PO, per os; PR, partial response; pt, patient; QW, once weekly; RSV, respiratory syncytial virus; SCID, severe combined immunodeficiency disorder; SOT, solid organ transplant; Tx, transplant; VR, virological response.

Multiple retrospective studies further examined the safety and efficacy of oral BCV for AdV treatment in comparison with CDV (Table 4). In a multicenter retrospective study by Hiwarkar et al. [77] with 41 pediatric and adolescent patients post-HSCT, 18 received BCV and 23 received CDV as preemptive treatment for AdV viremia. Virological response was observed in 83% of patients who received BCV compared to 9% in the CDV group. Additionally, nine out of eleven patients who did not respond to CDV had a virological response with BCV. BCV was stopped in one patient at 4 weeks due to severe abdominal cramps and diarrhea [77]. Perruccio et al. [79] reported 30 pediatric allogeneic HSCT patients with AdV reactivation (including 26 with AdV), totaling 44 episodes. CDV was used in 23 (52%) episodes as first-line treatment; BCV was used in 21 events, as first-line treatment in 7 (33%) and as rescue therapy in 14 (67%) after CDV failure. CDV treatment resulted in complete response in 35%, partial response in 4%, stable disease in 8%, and disease progression in 54% of cases. Whereas BCV treatment resulted in complete response in 48%, partial response in 9.5%, stable disease in 9.5%, and disease progression in 24%.

While these studies were limited by their retrospective nature, a phase 2 randomized placebo-controlled trial evaluated pre-emptive treatment with oral BCV for the prevention of AdV disease in pediatric and adult allogeneic HSCT recipients with asymptomatic AdV viremia [80]. Forty-eight subjects were randomized into three groups to receive either oral BCV 2 mg/kg twice weekly (BIW), BCV 4 mg/kg weekly (QW), or a placebo. After one week of therapy, undetectable AdV viremia was achieved in 67%, 29%, and 33% of patients in the BCV BIW, BCV QW, and placebo groups, respectively. Treatment failure rates were 21% for BCV BIW, 38% for BCV QW, and 33% for the placebo group. All-cause mortality was lower in the BCV BIW (14%) and BCV QW groups (31%) relative to the placebo group (39%), but not statistically significant. Diarrhea was the most common side effect reported in all three groups but led to treatment discontinuation in only one patient. Graft-versus-host disease (GVHD) of the GI tract was more common in the BCV BIW (50%) compared to the BCV QW (25%) and placebo (17%) groups. Despite its demonstrated antiviral activity against AdV, the development of oral BCV for AdV treatment has been hindered by significant GI side effects, particularly diarrhea and GVHD.

Another phase 2a study (NCT04706923) was conducted to assess the safety and efficacy of IV BCV for treatment of AdV. Preliminary results presented at the 2024 Tandem Meetings of the American Society of Transplantation and Cellular Therapy (ASTCT) and the Center for International Bone and Marrow Transplantation Research (CIBMTR) [81] demonstrated promising outcomes. From the experience of 27 immunocompromised patients, it was observed that 90% of patients achieved viral clearance in </= 4 weeks with IV BCV at a dose of 0.4 mg/kg twice weekly. Importantly, the GI and hepatic toxicities associated with oral BCV were not observed with IV BCV.

BCV has significantly enhanced oral bioavailability and reduced kidney toxicity compared to CDV. Retrospective studies suggest BCV is a viable alternative, particularly when CDV treatment fails [77,79]. Despite its efficacy, oral BCV use has been hindered by significant gastrointestinal (GI) side effects, including diarrhea and increased risk of GI graft-versus-host disease (GVHD) [80]. Unlike the oral formulation, IV BCV does not appear to cause severe GI and hepatic toxicities. Overall, these studies highlight the efficacy and improved safety profile of BCV, particularly in its IV formulation, as a promising alternative to CDV for the treatment of AdV [81].

4. Ribavirin (RBV)

RBV (1-β-D-ribofuranosyl-1,2,4-triazole-3- carboxamide) is a synthetic purine nucleoside analogue first synthesized in 1972 [82,83]. Unlike many other nucleoside or nucleotide analogues that exhibit antiviral activity by inhibiting viral nucleic acid replication, RBV

appears to have a much more diverse mechanism of action. As outlined by Graci and Cameron [84], direct mechanisms may include inhibition of RNA capping activity, inhibition of viral polymerases, and mutagenic effects via direct incorporation of RBV into newly synthesized viral genomes. Indirect effects include the reduction in cellular GTP pools via inhibition of inosine monophosphate dehydrogenase and an immunomodulatory role that promotes a T-helper type 1 immune response.

The aerosolized form of RBV was first FDA-approved for treatment of respiratory syncytial virus in 1986, and the oral form was licensed for treatment of chronic hepatitis C in 2003. Given its very broad-spectrum antiviral activities against both DNA and RNA viruses, it has been used off-label to treat a number of human viral pathogens with various degrees of success [85]. In many of these cases, the IV form of RBV, which was not FDA-approved, was employed as investigational treatment to treat serious viral infections under an EIND application [86].

Primary toxicity concerns of RBV are anemia and teratogenicity. RBV was shown in early animal and human cell models to suppress the release of erythrocyte precursors and reduce erythrocyte survival [87]. In clinical use, this has been best described in the treatment of hepatitis C infection in the era when RBV combined with interferon was the mainstay of treatment. RBV-associated anemia appears to be dose-dependent though reversible, and may be exacerbated by pre-existing anemia and renal dysfunction (likely due to increased RBV exposure) [88]. A variety of strategies have been successfully employed to mitigate RBV-associated anemia, including dose reduction as well as administration of epoetin alfa [89]. RBV has been shown to be teratogenic in hamster and rat models, and while human data is limited with unclear association, RBV exposure should be avoided during pregnancy and in the 6 months prior to pregnancy due to prolonged residence time in erythrocytes [90,91]. This presents concern not only for the patient but for healthcare workers as well. Aerosolized ribavirin requires personal protective equipment and negative pressure rooms due to potentially toxic ambient conditions surrounding RBV concentrations during administration. Additionally, all forms of ribavirin (oral and aerosolized) should not be handled by potentially pregnant healthcare workers [92,93].

The in vitro activity of RBV against AdV was first demonstrated in cell culture models over four decades ago [94,95]. Buchdahl et al. first described the successful use of nebulized RBV for the treatment of AdV pneumonia in two children (without any immunocompromised conditions reported) in 1985 [96]. The first case of successful treatment of AdV infection with IV RBV was reported in 1991 by Cassano et al. [97]. In that case, a 9-year-old male patient developed AdV-associated acute hemorrhagic cystitis following allogeneic HSCT. His symptoms failed to respond to vigorous hydration, diuresis, and analgesic therapy, but IV RBV produced rapid resolution of symptoms and AdV viruria.

Since its initial use, numerous reports have explored RBV as a treatment for AdV infection, yielding mixed outcomes. Some studies reported successful treatment [98–101], while others presented less promising results. Table 5 summarizes the outcomes of HSCT and SOT patients treated with RBV. For instance, Hromas et al. [102] reported four sequential allogeneic HSCT recipients with AdV infection treated with IV RBV, and all four failed to clear the AdV infection. Bordigoni [103] evaluated 35 HSCT patients with AdV infection, 18 of whom were treated with IV RBV. The authors concluded that RBV was ineffective, particularly in high-risk patients prone to disseminated disease. La Rosa et al. [104] reviewed 85 adult HSCT patients with AdV infection, including 12 treated with IV RBV, and found that RBV "was not associated with an appreciable benefit." In addition to clinical outcomes, Lankester et al. [105] prospectively measured quantitative AdV DNA load as a surrogate for treatment response. Among four pediatric allogeneic HSCT patients without immune recovery, RBV administration at the first signs of AdV dissemination did not reduce the AdV DNA load, with three patients showing increased viral loads. These collective findings cast doubt on the efficacy of RBV for treating severe AdV infections in immunocompromised patients.

Table 5. Transplant patients with adenovirus infection treated with ribavirin.

First Author [Reference]	Year of Publication	Number of Patients	Age/Sex	Underlying Condition	Onset of AdV from Tx	AdV Infection *	Species/ Serotype	RBV Route/Dose/ Duration (If Available)	Adjunctive Measure	Outcome **	Comments
HCT Cases:											
Cassano [97]	1991	1	9 Y/M	Allo-HCT (MRD) for AML	D + 36	Hemorrhagic cystitis	NA	IV; 33 mg/kg/d × 1 d, followed by 16.6 mg/kg/d × 8 d; daily dose divided into three doses given q8 h		Recovery	
Liles [106]	1993	1	25 Y/M	Allo-HCT (MMRD) for T-cell ALL	D + 75	Nephritis	B11	IV; 35 mg/kg/d × 1 d, followed by 25 mg/kg/d × 9 d; daily dose divided into three doses given q8 h	IVIG	Recovery	
Murphy [107]	1993	1	8 Y/M	Allo-HCT (Haploid) for acute nonlymphocytic leukemia	D + 103	Hemorrhagic cystitis	NA	IV; 35 mg/kg/d × 1 d, followed by 25 mg/kg/d × 8 d; daily dose divided into three doses given q8 h	/	Recovery	
Hromas [102]	1994	4	7 Y–45 Y	Allo-HCT (MUD) for NHL (1), MDS (1), ALL (2)	D + 20 to D + 147	GI; GU; GI + GU; disseminated	B11 (3 cases); A12 (1 case)	"Based on that recommended by Cassano [90]".—see above	IVIG (all)	Failure (all four pts), two died from AdV	
Jurado [108]	1995	1	27 Y/M	Allo-HCT (MRD) for aplastic anemia	D + 9	Hemorrhagic cystitis	B11	IV; 35 mg/kg/d × 1 d, followed by 25 mg/kg/d × 8 d; daily dose divided into three doses given q8 h	/	Recovery	
Kapelushnik [109]	1995	1	3 Y/M	Allo-HCT (MUD) for Wiscott-Aldrich syndrome	D + 45	Gastroenteritis	NA	IV; 30 mg/kg/d × 10 d; daily dose divided into three doses	IVIG	Recovery	
Wulffraat [110]	1995	1	8 M/M	Allo-HCT (Haploid) for SCID	D + 6	PNA, GI	NA	IV; loading dose 30 mg/kg; maintenance 15 mg/kg q6 h × 14 days	/	Recovery	
Mann [111]	1998	1	37 Y/F	Allo-HCT (MMRD) for AML	D + 33	Disseminated	NA	IV; 33 mg/kg q6 h × 5 d, followed by 16 mg/kg q6 h × 4 d, then 8 mg/kg q8 h × 1 d	/	Failure, died from AdV	
Chakrabarti [112]	1999	1	44 Y/M	Allo-HCT (MUD) for CML	D + 210	Hepatitis	NA	IV; loading dose of 35 mg/kg, followed by 25 mg/kg q8 h	/	Failure, died from AdV	

Table 5. *Cont.*

First Author [Reference]	Year of Publication	Number of Patients	Age/Sex	Underlying Condition	Onset of AdV from Tx	AdV Infection *	Species/ Serotype	RBV Route/Dose/ Duration (If Available)	Adjunctive Measure	Outcome **	Comments
Hale [113]	1999	2	Pediatric	HCT	NA	PNA; hemorrhagic cystitis	NA	IV	/	Failure, both died from AdV	
Howard [114]	1999	6	NA	Allo-HCT (partially matched)	NA	Hemorrhagic cystitis, nephritis	NA	IV	Some also received IVIG, but no detailed information available.	Failure	Thirteen pts were treated with RBV in this cohort, but results are available only for six pts.
				Allo-HCT (MUD)		GI, GU				Failure, died from AdV	
				HCT		URT, GI				Recovery	
				Allo-HCT (MRD)		Sputum, blood				Recovery	
			Adult	Auto-HCT		Hemorrhagic cystitis				Recovery	
			Pediatric	Allo-HCT (partially matched)		Hemorrhagic cystitis				Recovery	
Lakhani [115]	1999	1	26 Y/F	Allo-HCT (MUD) for CML	D + 38	Hemorrhagic cystitis	NA	PO; 1 g bid × 8 d	/	Recovery	AdV viruria resolved with RBV, but pt received E-aminocaproic acid to treat intractable hematuria.
Miyamura [116]	2000	8	11 Y–34 Y	HCT	NA	All with hemorrhagic cystitis except one also had PNA	NA	IV; For adults, 16 mg/kg q6 h × 4 d, followed by 8 mg/kg q8 h × 3 d. For children, 15 mg/kg/d × 10 d	/	Recovery (3); Failure (5), two died from ADV.	A total of nine pts in this cohort but one pt had early death from progression of underlying disease.
Bordigoni [103] ***	2001	13	Pediatric and adult	Allo-HCT [MUD (10); MRD (2); MMRD (1)]	D + 0 to D + 184	Definite (3); Probable (5); Asymptomatic (5)	NA	IV; loading dosage of 35 mg/kg followed by 25 mg/kg q8 h × 10 d.	/	Recovery (3), among one probable and two asymptomatic cases; Failure (10)	
La Rosa [104]	2001	12	18 Y–59 Y	NA	NA	PNA (2); hemorrhagic cystitis (1), enteritis (1), disseminated (8)	NA	IV	/	Recovery (2). But both with disseminated disease	

Table 5. *Cont.*

First Author [Reference]	Year of Publication	Number of Patients	Age/Sex	Underlying Condition	Onset of AdV from Tx	AdV Infection *	Species/ Serotype	RBV Route/Dose/ Duration (If Available)	Adjunctive Measure	Outcome **	Comments
Ikegame [117]	2001	1	50 Y/M	Allo-HCT (MMRD) for CML	D + 13	Disseminated	NA	PO; 1200 mg/d in divided doses × 4 d	IV vidarabine prior to RBV	Failure, died from AdV	
Gavin [118]	2002	1	18 Y/M	Allo-HCT (MUD) for AML	D + 15	Disseminated	B34	IV; 33 mg/kg on day 1, followed by 16 mg/kg q6 h × 3 d, then 8 mg/kg q8 h × 3 d	/	Failure, died from AdV	
Aebi [119] *	2003	1	41 Y/M	Allo-HCT (MUD) for ALL	D + 50	Hemorrhagic cystitis	B11	PO; 1st course: 16 mg/kg q6 h × 4 d, followed by 8 mg/kgq6 h × 3 d. 2nd course: 20 mg/kg q6 h × 5 d, followed by 10 mg/kg q6 h × 4 d.	/	Recovery	The pt had clinical improvement and reduction in AdV titer in urine after the 1st RBV course. When AdV titer increased and symptoms recurred 2–4 weeks later, the pt received a 2nd course of RBV, and responded well with resolution of AdV infection.
Omar [120]	2010	2	31 Y/F	Allo-HCT	D + 150	GI; viremia	C5	IV	/	Failure (2), both died from AdV	
			48 Y/M		D + 28	Disseminated	B35				
Sahu [121]	2016	1	36 Y/M	Allo-HCT for AML	D + 380	Hemorrhagic cystitis	NA	PO; 20 mg/kg in two divided doses × 4 weeks	Reduction in immunosuppression for GVHD	Recovery	
Takada [122]	2024	1	31 Y/M	HCT (cord-blood) for MPAL	D + 15	Hemorrhagic cystitis	NA	PO; 1200 mg/d × 44 d		Recovery	Received 5 days of GCV + vidarabine without effect before switching to RBV
SOT Cases:											
Arav-Boger	2000	1	13 M/F	Liever Tx ×2, 5 days apart	6 days from 2nd Tx	Hepatitis	C5	IV; Loading dose of 33 mg/kg, then 16 mg/kg q6 h × 4 d, followed by 8 mg/kg q8 h × 6 d	Reduction in immunosuppression	Recovery	Also received GCV and CMV IgG prior to RBV
Gavin [118]	2002	2	5 Y/F	Heart Tx	2 months post Tx	Cystitis, neprhitis	NA	IV; 25 mg/kg in three divided doses on day 1, then 15 mg/kg/d divided q8 h × 9 d		Failure (2), both died from AdV	
			2 M/M		5 weeks post Tx	PNA					

Table 5. *Cont.*

First Author [Reference]	Year of Publication	Number of Patients	Age/Sex	Underlying Condition	Onset of AdV from Tx	AdV Infection *	Species/ Serotype	RBV Route/Dose/ Duration (If Available)	Adjunctive Measure	Outcome **	Comments
Emovon [123]	2003	1	46 Y/F	Kidney and Pancreas Tx	22 months post tx	Hemorrhagic cystitis	NA	IV	Reduction in immunosuppression; IVIG	Recovery	
Hofland [124]	2004	1	60 Y/M	Liver Tx (1995), kidney Tx (2002)	2 moths post kidney tx	Nephritis, hemorrhagic cystitis	NA	NA; 400 mg bid × 3 weeks	High-dose prednisone	Recovery	
Komiya [125]	2009	1	63 Y/F	Kidney Tx	D + 7	Nephritis, hemorrhagic cystitis	B11	NA	IVIG; reduction in immunosuppression	Failure	
Park [126]	2015	1	32 Y/F	Kidney Tx	10 months post Tx	Nephritis, hemorrhagic cystitis, viremia	NA	NA; 400 mg bid × 3 weeks	IVIG; reduction in immunosuppression	Recovery	

* Disseminated infection is defined by involvement of two or more end-organs. Involvement of a single end-organ with viremia is not classified as disseminated disease. ** Failure is defined as lack of clinical and/or virologic response to treatment. For those patients with death reported, cause of death was attributed to AdV infection only for some. *** Pts that received other antiviral agent(s) with potential activities against AdV or donor leukocyte infusion in addition to ribavirin were excluded. **Abbreviations:** AdV, adenovirus; ALL; acute lymphocytic leukemia; Allo-HCT, allogeneic hematopoietic cell transplant; AML, acute myeloid leukemia; Auto-HCT, autologous hematopoietic cell transplant; CML, chronic myeloid leukemia; CMV, cytomegalovirus; GCV, ganciclovir; GI, gastrointestinal; GU, genitourinary; HCT, hematopoietic cell transplant; IgG, immunoglobulin; IV, IV; IVIG, IV immunoglobulin; MDS, myelodysplastic syndrome; MMRD, mismatched, related donor; MPAL, mixed-phenotype acute leukemia; MRD, matched related donor; MUD, matched unrelated donor; NA, not available; NHL, non-Hodgkin lymphoma; PNA, pneumonia; PO, per os; pt, patient; RBV, ribavirin; SCID, severe combined immunodeficiency; SOT, solid organ transplant; Tx, transplant; URT, upper respiratory tract.

More recently, the use of RBV in AdV has been reviewed by Ramfrez-Olivencia et al. [85], which included 21 isolated cases and seven case series from 1991 to 2017. These studies represented approximately 150 patients with AdV infection, though only about 60 individuals were treated with RBV (and some in combination with other antiviral agents). We conducted a focused review of cases involving HSCT and SOT recipients only, excluding those lacking sufficient data and those in which concomitant antiviral agents with potential AdV activity [such as CDV, ganciclovir (GCV), or vidarabine] were administered. As shown in Table 5, a total of 61 HSCT patients and seven SOT patients who received systemic RBV for AdV infection from 1991 to 2024 were included in our analysis. Of the HSCT cases, 22 cases (36.1%) reported successful outcomes. Among the successful cases, only four (18.2%) had disseminated disease, while the majority (59%) had AdV-associated hemorrhagic cystitis and/or nephritis. Conversely, of the 39 (63.9%) cases that resulted in treatment failure, 13 (33.3%) involved disseminated disease. The overall success rate for treating hemorrhagic cystitis and/or nephritis was 59.0%, while treatment success for disseminated disease was notably lower at only 23.5%. For the SOT patients, the overall success rate was 57.1%. Most of these patients had hemorrhagic cystitis, although one successful case involved the treatment of AdV hepatitis in a liver transplant recipient. Notably, spontaneous resolution of AdV viremia or mild end-organ disease, such as cystitis, may occur, particularly following engraftment in HSCT patients.

Despite focusing on transplant recipients, these cases still represented a highly heterogeneous population, including variations in transplant type, level of immunosuppression, severity of AdV infection, and so on. Given the discrepant results from these reports, there are several potential reasons for RBV's inconsistent efficacy against AdV. First, as previously discussed, AdV is not a single virus but comprises seven species of many serotypes/genotypes that can cause different clinical syndromes in various host populations and may differ in their sensitivity to RBV. In 2005, Morfin et al. [36] evaluated in vitro susceptibility of AdV to RBV and CDV using reference strains. They concluded that all tested serotypes were susceptible to CDV, whereas only species C serotypes were sensitive to RBV. However, in a subsequent study involving clinical isolates [127], RBV demonstrated activity against most isolates from species A, B, and D, as well as all species C isolates. Similarly, Stock et al. found that species C was more susceptible to RBV than other species [128]. Unfortunately, most cases listed in Table 5 do not report the AdV species or serotypes, limiting the ability to establish an association between species/serotypes and clinical outcomes. At present, AdV susceptibility to RBV cannot be reliably predicted based solely on species or serotype.

Second, another possible reason for RBV failure might be related to the concentration of RBV achievable at different sites of infection. The optimal dosing regimen for RBV in treating various forms of AdV infection remains undefined, which poses a challenge in clinical practice.

Third, for treatment of any infection, the extent of the disease and the timing of treatment initiation are of crucial importance. A trend toward better response was noted among patients with a single site of infection [114]. Not surprisingly, patients with disseminated infection had the poorest outcome per our analysis.

Finally, for most infections that can cause severe or life-threatening disease, the immune status of the hosts always plays a vital role in controlling disease progression and in recovery. Some have observed better efficacy of RBV among HSCT patients with siblings as donors as compared to other donors [116]. Patients with concomitant acute GVHD or a long delay between infection and treatment were found to be at greater risk of treatment failure [103].

Therefore, firm conclusions about RBV's efficacy for AdV treatment remain uncertain in the absence of prospective clinical trials. Further research is needed to clarify its role in the management of AdV infections.

5. Ganciclovir (GCV)

The activities of GCV [9-(1,3-dihydroxy-2-propoxymethyl guanine)] against different serotypes of AdV were demonstrated in 1988 using an in vitro plaque reduction assay [129]. The 50% effective dose (ED50) for AdV ranged from 4.5 to 33 μM, making it approximately 6 to 43 times less potent for AdV than for CMV. However, the drug concentrations achievable in patients receiving GCV for CMV treatment suggested potential efficacy against AdV infection. Subsequent in vitro and animal models also demonstrated GCV (or valganciclovir, VGCV)'s activity against AdV [130–132], but reports of its clinical use against AdV remain scant. In a systematic review by Gu et al. [133] that included 228 cases of AdV disease from 2000 to 2019, only 18 cases (7.9%) were treated with GCV. This was notably lower compared to other antiviral agents, with 36.0% of cases treated with CDV, 6.6% with RBV, and 5.3% with BCV.

The first case of successful AdV treatment with GCV was reported in 1992 with a renal transplant recipient who suffered from AdV-associated hemorrhagic cystitis [134]. Two other successful cases were then reported in 1997, including an HSCT patient with AdV-associated hemorrhagic cystitis and a cardiac transplant patient with severe AdV pneumonia [135,136]. A total of 21 cases using GCV for AdV treatment in HSCT or SOT recipients are found in the literature (Table 6). A majority (90%) of these cases reported successful outcomes. Among 16 HSCT or kidney transplant recipients presenting with nephritis or cystitis (including four with viremia and one with pneumonia concomitantly), treatment was successful in all but one case. However, for the four HCT patients with disseminated disease, only half survived despite treatment.

Table 6. Transplant patients with adenovirus infection treated with (val)ganciclovir.

First Author [Reference]	Year of Publication	Number of Patients	Age/Sex	Underlying Condition	Onset of AdV from Tx	AdV Infection *	Species/ Serotype	(V)GCV Route (If Available) **	Adjunctive Measure	Outcome ***	Comments
HCT Cases:											
Chen [135]	1997	1	47 Y/M	Allo-HCT (MRD) for AML	D + 52	Hemorrhagic cystitis	NA	IV GCV	/	Recovery	
Suzuki [137]	2008	1	35 Y/F	Allo-HCT (mismatched) for MDS	D + 24	Disseminated	B3 and B34	GCV		Recovery	
Nakazawa [138]	2009	1	8 Y/F	Allo-HCT (haploid) for AML	D + 24	Hemorrhagic cystitis	B11	IV GCV	/	Recovery	
Mochizuki [139]	2014	2	50 Y/M; 41 Y/F	Allo-HCT (MUD) for MM; Allo-HCT for AML	D + 20; D + 427	Disseminated (2)	NA	GCV		Failure, both died from AdV	
Yasuda [140]	2019	1	66 Y/F	Auto-HCT for MM	D + 46	Disseminated	NA	GCV	Discontinued of pomalidomide and dexamethasone	Recovery	GCV was started for CMV viremia, not intended for AdV
Takada [122]	2024	1	31 Y/M	HCT (cord-blood) for MPAL	D + 15	Hemorrhagic cystitis	NA	PO; 1200 mg/d × 44 d	Vidarabine	Failure	Received 5 days of GCV + vidarabine without effect, then switched to RBV
SOT Cases:											
Blohmé [134]	1992	1	28 Y/M	Kidney Tx	D + 25	Hemorrhagic cystitis	B7	IV GCV	Reduction in immunosuppression	Recovery	
Duggan [136]	1997	1	58 Y/F	Heart Tx	5 years from Tx	Pneumonia	NA	IV GCV	IVIG	Recovery	
Lim [141]	2005	1	51 Y/M	Kidney Tx	D + 36	Nephritis	NA	IV GCV followed by PO VGCV	Reduction in immunosuppression	Recovery	
Kozlowski [142]	2011	2	44 Y/M; 56 Y/M	Kidney Tx	A few days before D + 24; D + 19	Nephritis; one pt also had PNA	B34	PO VGCV	Reduction in immunosuppression	Recovery	Donor-derived from the same donor
Nanmoku [143]	2016	6	33–45 Y/ 2 F and 4 M	Kidney Tx	D + 7 to D + 1763	All with hemorrhagic cystitis; three pts also with nephritis and two of these with viremia	NA	IV GCV	Reduction in immunosuppression in three pts	Recovery (all 6)	Two pts also had BK viruria

Table 6. *Cont.*

First Author [Reference]	Year of Publication	Number of Patients	Age/Sex	Underlying Condition	Onset of AdV from Tx	AdV Infection *	Species/ Serotype	(V)GCV Route (If Available) **	Adjunctive Measure	Outcome ***	Comments
Paula [144]	2016	1	32 Y/M	Kidney Tx	1 month post Tx	Nephritis + viremia	NA	IV GCV	Reduction in immunosuppression	Recovery	
Barros Silva [145]	2017	1	38 Y/M	Kidney Tx	18 months post Tx	Nephritis + viremia	NA	GCV	IVIG; Reduction in immunosuppression	Recovery	
Moreira [146]	2019	1	40 Y/M	Kidney Tx	D + 17	Nephritis	NA	Iv GCV followed by PO VGCV	IVIG; Reduction in immunosuppression	Recovery	

* Disseminated infection is defined by involvement of two or more end-organs. Involvement of a single end-organ with viremia is not classified as disseminated disease. ** Dosing of IV GCV or oral VGCV were largely based on recommended dosing for CMV infection, with renal adjustment as needed. *** Failure is defined as lack of clinical and/or virologic response to treatment. For those patients with death reported, cause of death was attributed to AdV infection only for some. **Abbreviations:** AdV, adenovirus; Allo-HCT, allogeneic hematopoietic cell transplant; AML, acute myeloid leukemia; Auto-HCT, autologous hematopoietic cell transplant; CMV, cytomegalovirus; GCV, ganciclovir; HCT, hematopoietic cell transplant; IV, IV; IVIG, IV immunoglobulin; MDS, myelodysplastic syndrome; MM, multiple myeloma; MRD, matched related donor; MUD, matched unrelated donor; NA, not available; PO, per os; pt, patient; SOT, solid organ transplant; Tx, transplant; VGCV, valganciclovir.

The apparent success of GCV for the treatment of hemorrhagic cystitis and/or nephritis warrants further discussion. GCV is highly concentrated in the kidney tissues, and ~90% of the daily dose of GCV is excreted unchanged in the urine [147]. The GCV concentration in urine is also substantially higher than that in serum. For instance, in the case report by Nakazawa et al. [138], a 9-year-old girl who developed AdV-associated hemorrhagic cystitis after HSCT was successfully treated with GCV [138]. The authors noted that the peak serum concentration of GCV was 42.2 μM, but the concentration of GCV in urine exceeded 300 μM for nearly half a day after the infusion. Thus, despite the high EC_{50} of GCV for AdV, the very high drug concentration in urine might contribute to successful treatment outcome. Another piece of evidence suggesting potential clinical utility of GCV against AdV is based on the observation that HSCT patients receiving GCV for CMV prophylaxis had a lower risk for the development of AdV infection or for progressive AdV disease [148,149].

The major dose-limiting toxicity of GCV is hematologic toxicity, primarily neutropenia, occurring in more than 35% of patients receiving treatment-dose GCV therapy [150]. GCV-associated neutropenia is reversible in most cases and may be ameliorated with the administration of granulocyte colony-stimulating factor, permitting prolonged treatment [151].

Overall, the most robust data regarding the use of GCV in the treatment of AdV is primarily limited to cases of nephritis or cystitis. However, as previously discussed, hemorrhagic cystitis may resolve spontaneously without the need for specific antiviral treatment.

6. Other Anti-AdV Agents

Historically, vidarabine (AraA; 9-β-D-arabinofuranosyladenine) was also used clinically to treat AdV infection. It was the first FDA-approved nucleoside analogue to be administered systemically and was licensed in the United States in 1977 for the treatment of life-threatening HSV and VZV infections. However, given more favorable toxicity profiles of newer anti-herpes agents such as acyclovir, IV vidarabine was discontinued in the US in 2001, although it remains available as an ophthalmic ointment indicated for acute keratoconjunctivitis and recurrent epithelial keratitis secondary to HSV. Experience with this drug for AdV treatment is limited to only a handful of reports [11,103,122]. Most cases of success were for treatment of AdV-associated hemorrhagic cystitis [152–154] and pharmacokinetic data did support its possible efficacy in this setting [155].

Currently, there are no antiviral drugs approved for the treatment of AdV diseases despite their significant morbidity and mortality in vulnerable patient populations. However, there are continued efforts to identify effective anti-AdV therapies [156,157]. While various agents have demonstrated in vitro activities, none of these have been utilized for AdV treatment in clinical settings to date [158–160].

7. Adoptive T-Cell Therapy

As discussed above, conventional antiviral agents used for the treatment of AdV are limited by both unreliable efficacy and/or toxicity. The recognition that antiviral drugs were ineffective, especially for severe or disseminated AdV infections, highlights the need for immune-based approaches. Early research identified the critical role of T-cells in controlling AdV infections, particularly in immunocompromised individuals [51]. For instance, in a prospective study of renal transplant patients with AdV infection, an absolute lymphocyte count of <300 cells/μL was identified as a predictor of poor outcome, while an increase in virus-specific CD4+ and CD8+ T-cell counts was associated with successful viral clearance [161].

Adoptive T-cell therapy involves the transfer of virus-specific T-cells (VSTs) from a donor to a patient to enhance the immune response against infections. Donor lymphocyte infusions were initially used in the 1990s to treat viral infections in immunocompromised individuals [162,163]. However, these infusions often led to GVHD as a complication. Over the subsequent years, this field has evolved significantly with advancements in techniques for isolating and expanding T-cells, resulting in more effective and targeted therapies for AdV and other viral infections. A detailed description of the manufacturing of VSTs is below the scope of this review and has been well described in the literature [164].

The first step in the process of manufacturing VSTs involves selecting a donor. For HSCT patients, the cells can be obtained from the stem cell transplant donor or 3rd-party healthy donors. The safety and efficacy of donor-derived VSTs for AdV treatment have been evaluated in multiple studies (Table 7).

Table 7. List of studies using virus specific T-cells for treatment of AdV infection in HSCT patients.

Author [Reference]	Year	PMID/Clinical Trial No.	Study Description	No. of Patients (Total/with AdV)	Method of CTL Isolation/ Production	Results: Virological and /or Clinical Response	Results: Survival/Mortality Impact	Adverse Events
Studies using donor-derived VST's								
Leen [165]	2009	19700662/ NCR00590083	Clinical trial to assess the safety of cytotoxic T lymphocytes for prevention and treatment of EBV and AdV	12 received for prophylaxis and 1 for AdV Disease	Produced from donor PBMC's using AdV vector	Two patients with AdV disease cleared the infection; 11 patients who received ppx did not develop disease	/	None
Gerdemann [166]	2013	23783429/ NCT01070797	Phase 1/2 trial to study the safety and efficacy of VST's for AdV, CMV and EBV	10/5 AdV (n = 1) EBV + AdV (n = 2) CMV + AdV (n = 2)	DNA plasmids to generate donor-derived virus-directed T-cell lines with specificity for AdV, EBV, and CMV	Complete response in all five patients	/	None
Papadopoulou [167]	2014	24964991/ NCT01570283	Clinical trial assessing the feasibility and clinical utility of VSTs against EBV, AdV, CMV, BKV, HHV-6	11 /1	Rapidly-generated single-culture VSTs that recognize 12 immunogenic antigens from five viruses using allogeneic stem cell donor	94% with complete virological response; one AdV infection resolved	Two died due to non-infectious causes	Skin GVHD in one patient
Feucht [168]	2015	25617426/ 2005-001092-35 EU Clinical trial register	Clinical trial to analyze the safety and efficacy of ex vivo adoptive T-cell transfer for AdV	30/30	PBMCs were isolated from stem cell donors, after being stimulated by hexon protein	86% with complete clearance of viremia (Responders)	71% (15) of responders survived; 100% (eight out of eight) non-responders died. Attributable mortality due to AdV is 100% in non-responders and 9.5% responders.	Mild GVHD grade 1 in two patients within 2 weeks after ACT and GVHD grade 2–3 in four patients > 7 weeks after ACT
Creidy [169]	2016	27246524/ NCT01325636	French multicenter pilot trial to treat peds/adult patients post-HSCT with CMV/AdV infection with VST's	15/8 5 AdV 3 AdV + CMV	Donor cells were stimulated with hexon AdV antigen followed by magnetic enrichment of IFN-γ–secreting cells using the Cytokine secretion system and the CliniMACS device	Of the five patients alive, four showed a complete virological response and one was a no response	Two died prior to 21 day evaluation and one death was attributable to AdV	Four patients with respiratory failure (unclear if it was related to AdV or ACT) and one patient developed GVHD but unclear if associated with ACT
IP, [170]	2018	29753677/ NCT01822093	Open-label phase 1/2 study to assess the safety of pre-emptive administration of AdV-specific T-cells to treatment AdV viremia in high-risk pediatric patients after HSCT.	8/8	AdV-specific T-cells were expanded from donors using peptides and cytokines	All eight patients with complete virological response	Two deaths (one due to AdV infection despite clearance of viremia)	Grade 4 GVHD in one patient

Table 7. *Cont.*

Author [Reference]	Year	PMID/Clinical Trial No.	Study Description	No. of Patients (Total/with AdV)	Method of CTL Isolation/ Production	Results: Virological and /or Clinical Response	Results: Survival/Mortality Impact	Adverse Events
Abraham [171]	2019	31292125/ NCT0880789 NCT01923766	Clinical trial to evaluate the feasibility and safety of CB-derived multivirus-specific T-cells in pediatric patients	14/1	PBMCs from cord blood donors were used to generate LCLs and dendritic cells, then transduced with AdV vector or with peptide mix	All who received CB-VSTs as ppx did not develop any end-organ disease from CMV, EBV, or AdV; one patient with AdV disease resolved	Two died; not attributable to viral infection	Grade 3 GI GVHD in one patient
Rubinstein [172]	2022	35108727/ NCT03883906	Single-arm, phase 2 study to assess the efficacy of donor-derived VST's in prevention of viral infection due to CMV, AdV, BKV and EBV	23/NA	PBMC's were stimulated with peptide mixes	21% (five) with treatment failures; three developed significant viremia/viral disease requiring additional antiviral therapy, one due to AdV.	Four deaths; not attributable to viral infection	GVHD in two patients
	Studies using 3rd-party VST's							
Leen [173]	2013	23610374/ NCT00711035	Multicenter study of 3rd-party VSTs to treat CMV, EBV and AdV post-HSCT	50/18	PBMCs were transduced with Ad5f35pp65 vector	6-week cumulative response rate of 77.8% (95% CI, 53.7–100%) for AdV	Five deaths; all attributable to AdV	Two patients with grade 1 GVHD de novo
Tzannou [174]	2017	28783452/ NCT02108522	Phase 2 clinical trial using off the shelf T-cells for treatment of multiple viruses (CMV, AdV, EBV, BKV, HHV6) post-HSCT	38/7	Posoleucel—3rd-party VSTs generated using peptide multimers	four CR; one PR; two non-response (Cumulative response rate of 71.4%)		Recurrent grade 3 GI GVHD in one patient and grade 1–2 skin GVHD in five patients
Pfieffer [175]	2023	36628536/ NCT02108522	Open-label, phase 2 trial to determine the feasibility and safety of posoleucel in HSCT recipients with AdV, BK virus, CMV, EBV, HHV-6, and JC virus.	58/12	Posoleucel—3rd-party VSTs generated using peptide multimers	6-week response was observed in 10 of 12 patients (83%; 95% CI, 51.6–97.9%),	/	13/58 (22%) patients developed GVHD
Keller [176]	2024	38637498/ NCT03475212	Phase 2 multicenter study using partially-HLA matched VSTs targeting CMV, EBV or AdV	51/30 24 AdV 6 CMV + AdV	/	Virological response in 74% (17/23)	Overall survival 57.1% (95% CI: 42.00–70.00%) at 1 year	Grade III cytokine release syndrome occurred in one patient requiring treatment with tocilizumab and steroids. Graft rejection in one patient associated with infusion

Table 7. *Cont.*

Author [Reference]	Year	PMID/Clinical Trial No.	Study Description	No. of Patients (Total/with AdV)	Method of CTL Isolation/ Production	Results: Virological and /or Clinical Response	Results: Survival/Mortality Impact	Adverse Events
Studies using both donor- derived and 3rd-party VST's								
Qian [177]	2017	2848908/ NCT0285157	Phase 1/2 multicenter pilot study involving the infusion of AdV-VST after HSCT in the event of refractory ADV infection or disease.	11/11	AdV-VST generated by interferon (IFN)-γ-based immunomagnetic isolation from their original donor (42.9%) or a third-party haploidentical donor (57.1%)	Virological response in 91% patients	four deaths; one attributable to AdV	GVHD in three patients
Rubenstein [55]	2021	34473237/ NCT02048332 NCT02532452	Single-center phase 1/2 clinical trial to assess safety and efficacy of VSTs for treatment of adenoviremia	29/29 7 DD 21 TP 2 both	PBMCs were stimulated with pools of viral peptides (Pepmix) encompassing antigen epitopes.	Clinical response in 81%, with a CR in 58%; CR and overall response rates were higher in patients treated with DD VSTs compared with TP (86% vs. 42% CR; 74% vs. 100% overall response)	/	GVHD in one patient who received 3rd-party VSTs
Galletta [178]	2023	36736781/ NCT02532452 NCT02048332	Retrospective cohort study of patients who received VSTs for treatment of AdV, BKV, CMV and EBV	145/37 77 DD 68 TP	PBMCs were stimulated with pools of viral peptides (Pepmix) encompassing antigen epitopes.	Clinical response rate for AdV was 64.9%. No difference in outcomes between DD and TP	81% survived in DD and 66% in TP group after 1 year	Eight and five patients from DD and TP donors developed GVHD

Abbreviations: ACT; adoptive T-cell therapy, AdV; adenovirus, BKV; BK polyomavirus, CB; cord blood, CMV; cytomegalovirus, CR; complete virological response, DD; donor-derived, EBV; Epstein–Barr virus, GI, gastrointestinal; GVHD; graft-versus-host disease, HLA; human leukocyte antigen, HHV-6; human herpesvirus 6, HSCT; hematopoietic stem cell transplant; IFN; interferon, LCL; lymphoblastoid cell line, PBMC; peripheral blood mononuclear cell, ppx; PR, partial virological response; prophylaxis, TP; third party, VSTs: virus specific T-cells.

Virus-specific T-cell therapy has also been used to treat refractory infections in SOT patients [179]. In 2006, Leen et al. demonstrated that multivirus-specific T-cells targeting CMV, EBV, and AdV (derived from a single culture and expanded in patients) led to reductions in viral titers and resolution of associated symptoms [180]. Additionally, the safety of EBV- and AdV-specific T-cells was shown in 20 pediatric patients who had undergone haploidentical or matched unrelated donor transplants [165]. None of these patients developed EBV proliferative disease, and two had resolution of AdV infection without any reported GVHD. Similarly, a clinical trial in Germany evaluated the safety and efficacy of hexon-specific T-cell therapy for AdV infections. Among 14 patients, VSTs induced in vivo antiviral immunity lasting up to six months, with viral control leading to complete clearance of viremia in 86% of patients with antigen-specific T-cell responses. Six-month survival was markedly higher in responders compared to non-responders, who all died shortly after adoptive T-cell therapy. GVHD grade 1 occurred in two patients within 2 weeks and grade 2–3 in four patients at approximately seven weeks after VST administration. Although the late onset of GVHD suggested other possible causes, the role of VSTs could not be definitively excluded [168]. Ip et al. conducted a phase 1/2 open-label trial to evaluate the safety and efficacy of AdV-specific T-cells in high-risk pediatric patients. All eight patients cleared viremia between days 56 and 127. AdV-specific T-cells were detectable until day 90 in all patients via ELISpot assay. However, one patient developed GVHD requiring steroid treatment, which led to AdV reactivation, respiratory failure, and death [170]. The efficacy of VSTs in preventing viral infections in immunocompromised patients has also been assessed. In a clinical trial by Rubinstein et al. [172], 23 patients received VSTs targeting CMV, AdV, BKV, and EBV on day 21 post-transplantation to assess their efficacy in preventing viral infections. Of these, 18 did not develop infections, 2 patients experienced EBV viremia, 1 developed symptomatic BK viruria, 1 developed CMV viremia, and 2 developed clinically significant GVHD. While this study demonstrated effectiveness, it was limited by its small sample size and lack of a control arm [172].

Overall, these studies have demonstrated the safety and efficacy of donor-derived virus-specific T-cells, with significant reductions in viral loads, resolution of symptoms, and minimal GVHD in some trials. Nevertheless, challenges such as late-onset GVHD, small sample sizes, and the absence of control arms in many trials are some of the limitations.

Despite the successful use of adoptive T-cell therapy in many of these cases, the generation of VSTs for each individual patient from the stem cell donor is a time-consuming process and requires the donor to be seropositive for AdV. It may also increase the risk of GVHD if the cells are directly isolated from donor leukocytes using methods like antigen capture or using viral peptides. Furthermore, this approach may not be practical for urgent or widespread use. These limitations led to exploration of the use of 3rd-party healthy donors to generate VSTs [175,181]. Third-party VSTs can be partially HLA-matched and may be used for multiple recipients. However, donor-derived VSTs may have longer persistence due to a higher degree of human leukocyte antigen (HLA) matching compared to third-party VSTs.

In 2013, Leen et al. generated a third-party bank of VSTs and conducted a multicenter clinical trial to assess their efficacy [173]. The third-party VSTs were matched at one HLA allele. Among 50 patients, 18 had AdV infections. Of these, 77% experienced partial or complete responses within six weeks post-infusion. Across the entire cohort, two patients developed de novo GVHD, but no other toxicities were reported.

To identify potential donors for T-cell therapy, Li Pira et al. [182] measured donor T-cell responses to different viral antigens using a cell-ELISA assay. They demonstrated a strong correlation between the frequency of specific T-cells and the cell-ELISA results, which is useful for selecting the best donors. Based on their findings, they advocate for the

creation of registries for third-party donors who are HLA-typed and fully characterized for pathogen-specific T-cell immunity. This approach would expand the use of third-party donors and increase the likelihood of better HLA matching.

In 2021, the FDA granted orphan drug designation to Posoleucel, a multivirus-specific T-cell therapy derived from partially HLA-matched third-party donors. Posoleucel was designed to prevent or treat multiple viruses, including AdV, BKV, CMV, EBV, HHV-6, and JC virus. In an open-label, single-arm phase 2 study, 59 cell lines were administered to 58 patients [175]. Overall, 55 out of 59 patients showed partial or complete responses at six weeks post-infusion. Among the 12 patients with AdV infections, 19 infusions were administered, with a response rate of 83% (10 of 12) observed by week 6. Thirteen of 58 patients (22%) developed GVHD. This is one of the largest reported trials demonstrating the safety and efficacy of third-party VSTs for the treatment of viral infections.

The promising results from the above trial prompted another phase 2 open-label single-arm study [183] (NCT04693637) to evaluate the safety and efficacy of posoleucel in preventing six viral infections. Of the 26 patients enrolled, 3 (12%) had clinically significant infection after 14 weeks, and 5 (19%) patients had grade 2–4 GVHD. T-cell responses persisted until week 14 as measured by deep sequencing. Six patients died due to disease relapse or progression. This study was limited by its small sample size, the absence of a comparison group, and the fact that the posoleucel infusion was administered relatively late, with a median of 42 days after HSCT [183]. In the phase 3 portion of this trial (NCT05305040), 377 patients were enrolled; however, the study was terminated early in December 2023 due to futility. No safety concerns were identified. Another randomized, placebo-controlled trial investigated the use of posoleucel for treating AdV infections in pediatric and adult patients following HSCT. However, this trial was terminated early due to its failure to meet the predefined endpoint (NCT05179057).

There are very few studies that have compared the efficacy of donor-derived vs. third-party VSTs (Table 7). The largest study reported involved 145 children at Cincinnati Children's Hospital who received VSTs to treat AdV, BK virus, CMV, and/or EBV. This retrospective study [178] compared the clinical efficacy and safety outcomes of donor-derived VSTs and third-party VSTs. No statistically significant differences were observed in clinical response rates between the donor-derived and third-party cohorts (65.6% versus 62.7%), incidence of GVHD, or overall survival at 30, 100 days, and one year post-transplantation.

In their review, O'Reilly et al. [181] describe the experiences of multiple centers in creating banks of varying sizes containing EBV-, AdV-, and CMV-specific cell lines. The advantage of these banks is that the cells are readily available for use. They are characterized by their HLA types, which allows for the selection of appropriate HLA-restricted T-cells for patient treatment [181]. However, the feasibility of maintaining and implementing these banks may pose challenges for many centers.

VSTs have shown significant efficacy in treating AdV and other viral infections in immunocompromised patients, with viral clearance rates of 77–94% reported in clinical trials. While VST therapy is generally well-tolerated, for HSCT recipients, GVHD remains a notable complication. Currently, the widespread adoption of VST therapy is hindered by the lack of randomized controlled trials to comprehensively assess its safety and efficacy, as well as the complexity of its manufacturing process. Future studies with larger cohorts, robust designs, and standardized endpoints are essential to establish its clinical utility. Additionally, CRISPR technology is being leveraged to enhance the precision and efficacy of VSTs by improving specificity, minimizing off-target effects, and engineering resistance to viral immune evasion mechanisms [184].

8. Conclusions

AdV infections pose a major clinical challenge, especially in immunocompromised populations, resulting in significant morbidity and mortality. Currently, CDV remains the primary antiviral agent of choice despite its modest efficacy and dose-limiting nephrotoxicity. BCV offers a promising alternative with a more favorable safety profile, particularly in its IV formulation. RBV and GCV have demonstrated mixed efficacy, exhibiting variable responses across different clinical diseases and possibly depending on specific AdV serotypes/genotypes. Adoptive T-cell therapy has emerged as a transformative approach for managing severe and refractory AdV infections, providing enhanced viral clearance and improved clinical outcomes. However, challenges such as the complexity of T-cell manufacturing and the risk for GVHD remain barriers to widespread adoption. Future research should prioritize the development of standardized treatment protocols and the conduct of robust comparative studies to determine optimal dosing regimens and the timing for initiating treatment. Additionally, therapies must be tailored to individual patients. There is a pressing need for ongoing research into safer and more effective therapeutic options, including novel antiviral agents and immune-based therapies, to improve patient outcomes in the management of severe AdV infections.

Author Contributions: Conceptualization: D.Y.H. and N.N.; data curation: N.N., D.H. and D.Y.H.; writing: N.N., D.H. and D.Y.H.; writing—review and editing: N.N., D.H. and D.Y.H.; supervision: D.Y.H. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Conflicts of Interest: The authors declare no conflicts of interest.

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viruses

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Review

HBV Reactivation in Immunosuppressed Patients: Screening, Prevention, and Management Including Solid Organ Transplant Recipients

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Abstract: Hepatitis B virus (HBV) infection remains a global health challenge, affecting over 254 million individuals chronically and contributing significantly to cirrhosis, liver failure, and hepatocellular carcinoma. Despite advancements in antiviral therapy, HBV reactivation remains a critical concern, particularly in immunosuppressed individuals, including non-transplant patients undergoing immunosuppressive therapy and solid organ transplant recipients. This review provides screening and management strategies for HBV reactivation in these populations.

Keywords: hepatitis B; de novo infection; immunosuppression; chronic hepatitis B infection

1. Introduction

Globally, more than 2 billion people are estimated to have been exposed to the hepatitis B virus (HBV), and approximately 254 million are chronically infected, making chronic hepatitis B (CHB) one of the leading causes of cirrhosis, liver failure, and hepatocellular carcinoma (HCC) [1]. Viral suppression with nucleos(t)ide analogs (NA) dramatically reduces the risks of these adverse liver-related outcomes, yet there are currently no curative therapies [2]. As a result, individuals with chronic hepatitis B (HBV surface antigen [HBsAg] positive) and those who were previously infected (HBsAg negative/core antibody [anti-HBcAb] positive) remain at risk for HBV reactivation [3]. Although HBV reactivation can occur spontaneously, this risk is significantly increased with exposure to immunosuppressive therapies. In this review, we will provide an overview of managing and mitigating the risk of HBV reactivation in two clinical scenarios: (1) non-transplant patients on immunosuppressive medications and (2) solid organ transplant recipients.

2. The Pathophysiology and Clinical Course of HBV Reactivation

HBV is a hepatotropic virus that can be transmitted via blood, semen, and other body fluids from an infected individual [1,4]. In patients with chronic HBV infection, viral control is the result of a complex interaction between virologic factors and host immunity. While many patients with chronic HBV infection may have an immune-active disease warranting antiviral treatment, some may have an inactive disease (e.g., low HBV DNA level, normal liver enzymes, and histology), which portends a low risk of disease progression; thus, long-term antiviral treatment is not recommended [2,5]. However, these

patients with immune-inactive chronic HBV infection may still reactivate with a rapid rise in HBV DNA levels and/or liver enzymes either spontaneously or with immunosuppressive therapy [6–9]. For those with CHB (HBsAg positive), HBV reactivation is defined as any one of the following: (1) a 100-fold increase in HBV DNA compared to the baseline level; (2) HBV DNA $\geq$ 10,000 IU/mL if the baseline HBV DNA level is unknown; or (3) HBV DNA $\geq$ 1000 IU/mL in a patient with a previously undetectable level [10].

Even those with resolved HBV infection are at risk for HBV reactivation due to the persistence of covalently closed circular DNA (cccDNA) formed within the nucleus of the hepatocytes previously infected with HBV, which provides a template for future HBV replication [11]. These patients can thus be described as having "latent HBV", as indicated by an HBsAg-negative/HBVcAb-positive serostatus. For patients with latent HBV, HBV reactivation is defined by de novo HBV replication as confirmed by new HBsAg positivity and/or a newly detectable HBV viral load [12].

Once HBV reactivation occurs, the clinical course varies considerably, but some will experience life-threatening complications, including liver failure, with high mortality since many of these patients taking immunosuppressive therapies are not candidates for rescue liver transplant due to their oncologic or non-oncologic comorbidities. HBV reactivation in the context of immunosuppression presents initially with a marked increase in HBV DNA levels. This phase can start as early as within a few weeks after immunosuppression initiation, and patients are often asymptomatic. Among those with HBV reactivation, up to 40% will develop HBV reactivation-related hepatitis [6,7,13], as characterized by elevated alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels. This hepatitis phase lags behind the HBV replication phase by several weeks. Although not all patients who experience HBV reactivation will develop reactivation-related hepatitis, the presentation can be severe, manifesting in jaundice, liver synthetic dysfunction, and even liver failure requiring salvage liver transplantation despite the use of NA therapy [14]. Furthermore, late reactivation has been described as occurring even beyond 12 months after cessation of immunosuppression due to a delay in immune reconstitution [8].

3. Screening and Testing for HBV

Professional society management guidelines from a variety of medical subspecialties have uniformly recommended HBV screening (HBsAg and anti-HBc) prior to initiating immunosuppressive therapy (Figure 1) [2,5,10,12]. However, universal HBV screening inclusive of HBsAg, anti-HBc, and anti-HBs has been recommended by the U.S. Centers for Disease Control (CDC) since March 2024 for all U.S. adults [15]. A positive serologic marker (HBsAg and/or anti-HBc) should be followed up with an HBV DNA viral level. Hepatitis B surface antibodies can also be assessed, and patients are offered vaccination if non-immune. In clinical practice, patients who are identified to have CHB (HBsAg positive) should undergo a complete evaluation, including baseline HBV viral load, ALT level, HBeAg and HBeAb status, serologic evaluation for co-infections (HIV, HCV, HDV), and an assessment of underlying fibrosis to determine any indications to start NA therapy regardless of any planned immunosuppressive therapy. The indication for antiviral therapy in CHB outside the setting of immunosuppressive therapy is beyond the scope of this review but has been addressed by practice guidelines [2,10].

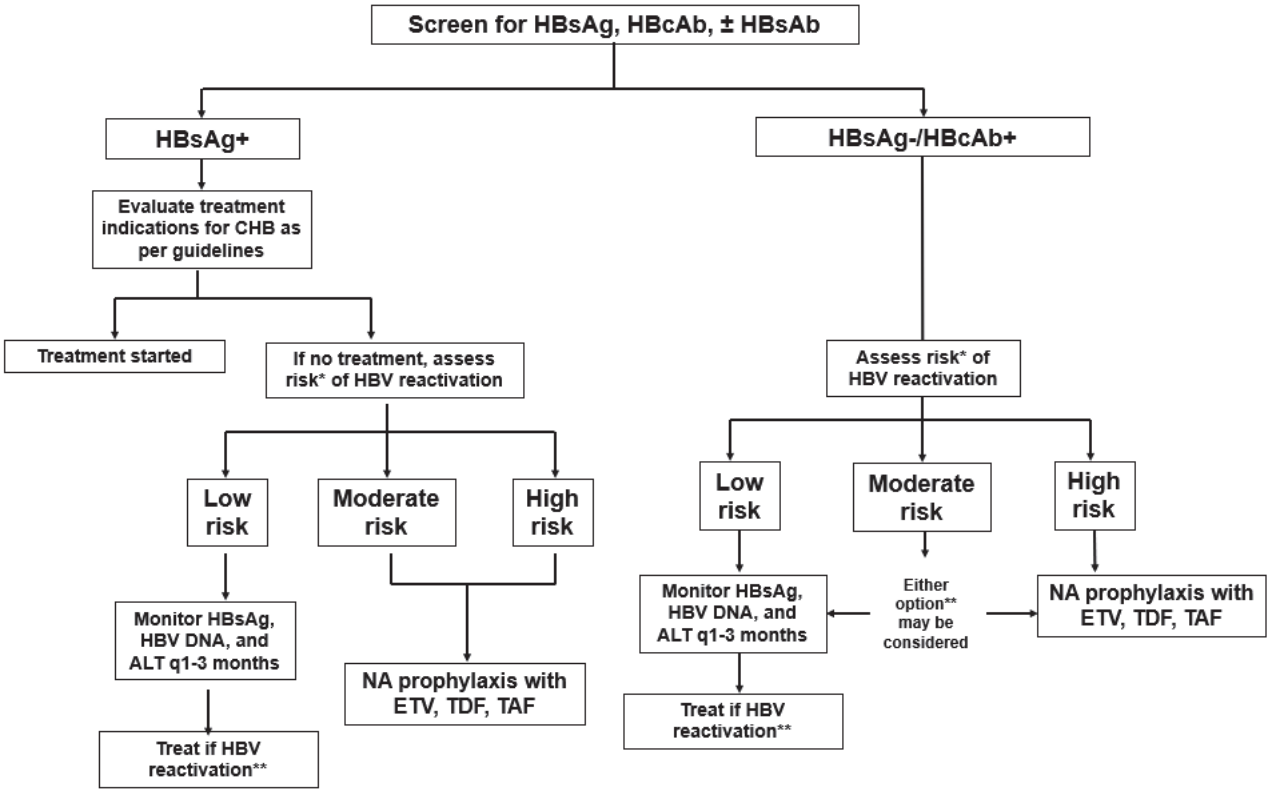

Figure 1. Algorithm for the management of HBV reactivation based on HBV serology and risk categories for patients receiving immunosuppression. * HBV risk is defined as (1) low risk: <1% risk of reactivation; (2) moderate risk: 1–10% risk of reactivation; (3) high risk: >10% risk of reactivation. ** NA treatment should consist of entecavir (ETV), tenofovir disproxil (TDF), and tenofovir alafenamide (TAF).

Despite these recommendations, real-world screening rates are suboptimal and highlight a critical deficiency in HBV screening prior to the initiation of immunosuppressive therapies. In a U.S. multi-institutional study of 11,959 oncologic patients who were planning to receive immunosuppressive therapies as part of their cancer treatments, only 2045 (17.1%) were screened for either HBsAg or anti-HBc [16]. Among those tested, 0.9% had CHB (HBsAg positive), and 8.4% were previously exposed (HBsAg negative/anti-HBc positive). In another cross-sectional study of national claims data from Japan, among 82,282 patients with rheumatoid arthritis, only 9.7% had received the appropriate HBV screening prior to initiation of immunosuppressive therapy [17]. These findings highlight a critical deficiency in HBV screening practices and a need for patient and provider education, as well as changes in system practice.

In addition to conventional HBV serologies and standard HBV DNA PCR testing, several newer HBV biomarkers are available for use. Ultra-sensitive HBV DNA [18] testing may detect very low-level viremia (beyond standard HBV DNA testing), which may help to identify those at higher risk for reactivation. Quantitative HBsAg (qHBsAg) [19] has also been found to be more sensitive than standard HBV DNA in reflecting underlying HBV disease activity. Other emerging biomarkers include hepatitis B core-related antigen [20], HBV pregenomic ribonucleic acid [21] (both may serve as surrogates for cccDNA activity), and quantitative anti-HBc [22] (lower levels may be associated with a higher risk of HBV recurrence after liver transplantation). It remains unclear whether these biomarkers provide significant incremental benefits over routine HBV serologic testing and then standard HBV DNA monitoring in most clinical scenarios.

4. Risk Factors for HBV Reactivation and Clinical Presentation

HBV risk stratification is crucial for guiding subsequent management strategies. A key determinant of reactivation risk is the HBV serologic status: patients with CHB (HBsAg positive) have a significantly higher risk of reactivation compared to those with latent HBV (HBsAg negative/anti-HBc positive) [12,23]. For example, a meta-analysis of 29 studies involving 1409 HBV-infected patients found that the prevalence of HBV reactivation among CHB patients receiving biologic therapies ranged from 17.1% to 40.5% [24]. By contrast, the reactivation rate among those with occult HBV infection ranged from 2.6% to 6.4%.

Consequently, subsequent management strategies are further tailored according to whether the patient has CHB or latent HBV. Within each HBV status, patients can be categorized into three risk categories for reactivation and with additional recommendations for management changes: low risk (<1%), moderate risk (1–10%), and high risk (>10%) for reactivation (Figure 1) [5,12,14]. Beyond HBsAg and anti-HBc status, other virologic characteristics associated with increased HBV reactivation risk include high baseline viral load [25], non-A HBV genotype [26], HBeAg seropositivity [25], and the absence of anti-HBs among patients with latent HBV [27].

4.1. Risk Stratification According to Type of Immunosuppression

Risk stratification is further guided by the type of immunosuppressive therapy that is administered (Figure 1, Table 1). One of the most well-characterized is B-lymphocyte-depleting therapies, such as rituximab, which carries a "black box" warning for HBV reactivation. Rituximab is an anti-CD20 monoclonal antibody rituximab which depletes B-lymphocytes through immune-mediated mechanisms. For both patients with CHB and latent HBV, the risk of reactivation has been consistently shown to be within the "high risk" (>10%) category [9,28]. In addition, a substantial proportion of HBV reactivation occurs even 12 months after discontinuation of rituximab, presumably due to delayed immune recovery [28,29]. As such, major societies have advised continuing NA therapy for 12–18 months after the completion of rituximab or similar agents (i.e., rituximab, ofatumumab, obinutuzumab) [2,5,10,12].

Table 1. HBV reactivation risk according to HBV serologic status and type of immunosuppression *.

HBV Status	HBsAg Positive (CHB)	HBsAg Negative/anti-HBc Positive (Latent HBV)
High risk (>10%)	- Anti-CD20 monoclonal antibodies: rituximab, ofatumumab, obinutuzumab - Moderate to high dose corticosteroids (prednisone $\geq$ 10 mg/day for $\geq$4 weeks) - Anthracycline derivatives - TNF-alpha inhibitors - Immune checkpoint inhibitors - HCV co-infection undergoing direct-acting antiviral therapy - Tyrosine-kinase inhibitors including imatinib and nilotinib	- Anti-CD20 monoclonal antibodies: rituximab, ofatumumab, obinutuzumab

Table 1. *Cont.*

HBV Status	HBsAg Positive (CHB)	HBsAg Negative/anti-HBc Positive (Latent HBV)
Moderate risk (1–10%)	- Calcineurin inhibitors - Low-dose corticosteroid use (prednisone < 10 mg/day $\geq$ 4 weeks) - Cytotoxic chemotherapy	- Moderate to high dose corticosteroids (prednisone $\geq$ 10 mg/day for $\geq$4 weeks) - Anthracycline derivatives - Cytotoxic chemotherapy - Calcineurin inhibitors - Tyrosine-kinase inhibitors including imatinib and nilotinib
Low risk of reactivation (<1%)	- Antimetabolites such as azathioprine, 6-MP, methotrexate - Short-term corticosteroids (<1 week) of any dose - Intra-articular steroid injections	- TNF-alpha inhibitors - Antimetabolites, azathioprine, 6-MP, MTX - Low-dose corticosteroids (prednisone <10 mg) - Short-term corticosteroids (<1 week) of any dose - Intra-articular steroid injections - HCV co-infection undergoing direct-acting antiviral therapy

* Adapted with permission from systematic review and guidelines [3,30].

Therapies associated with a low risk of reactivation for patients with either CHB or latent HBV include brief (<1 week) courses of corticosteroids, antimetabolites/antiproliferative agents (e.g., azathioprine, 6-mercaptopurine, methotrexate, mycophenolate mofetil), and intra-articular steroid injections. Other therapies including high-dose corticosteroids, anthracyclines, TNF-alpha inhibitors, cytotoxic chemotherapies, and calcineurin inhibitors are associated with either a moderate (1–10%) or high risk (>10%) of HBV reactivation, depending on whether they are used in patients with CHB or latent HBV. We refer the readers to other publications for a more detailed discussion regarding HBV reactivation risk associated with each type of immunosuppressive therapy [5,12,31,32].

4.2. Patient and Disease Characteristics Associated with HBV Reactivation

In addition to HBV serology and the type of immunosuppressive treatment, several patient-related factors can also impact the risk of HBV reactivation. Patient characteristics associated with a higher risk of HBV reactivation include male sex, older age, advanced fibrosis, and hematologic (vs. non-hematologic) disorders. Among patients with cirrhosis, the risk of HBV reactivation is notably higher (ref), and the consequences are more severe. Cirrhotic patients face increased rates of hepatitis, liver failure, and mortality should HBV reactivation occur. A meta-analysis performed by Cholongitas et al. found that patients with hematologic malignancies were at a higher risk of HBV reactivation (10.9%) when compared to patients with non-hematologic diseases (3.6%) [23].

Co-infection with other hepatotropic or non-hepatotropic viruses can pose special clinical scenarios. In patients with chronic hepatitis C virus (HCV) infection and HBV co-infection, the use of direct-acting antiviral therapy has been linked to the risk of HBV reactivation. A recently published systematic review found that the baseline risk of reactivation was low (2 per 1000) for patients with latent HBV but high (240 per 1000) for patients with CHB [3]. Co-infection with HBV/human immunodeficiency virus (HIV) is also commonly seen due to the shared routes of transmission. Current guidelines recommend initiating antiretroviral therapy in all HIV-infected individuals—regardless of CD4 count—which includes agents with anti-HBV activity, typically tenofovir (TDF or

TAF) plus emtricitabine or lamivudine [33]. Treatment interruptions should be avoided, as they can lead to HBV reactivation and severe hepatitis. If a change in the HIV/HBV regimen is required due to HIV virologic failure or other reasons, HBV-active agents must be maintained or replaced with equally potent alternatives, considering potential HBV cross-resistance (e.g., prior LAM exposure can lead to an increased risk of ETV resistance). For more comprehensive discussions on managing HIV/HBV co-infection, readers are referred to published guidelines and reviews on this topic [34].

5. Risk-Stratified Approach to Managing HBV Reactivation

Two management strategies are employed to manage the risk of HBV reactivation. The first strategy is to initiate NA prophylactic therapy during and for 6–12 months following the end of immunosuppressive treatments. The second strategy is to monitor HBV studies (ALT, HBsAg, HBV DNA every 3 months) and provide "on-demand" NA therapy at the first sign of HBV reactivation. When deciding between antiviral prophylaxis versus monitoring, shared decisions and discussions should be made between patients and providers to realistically evaluate patient ability and willingness to maintain regular long-term monitoring, as often required for patients receiving immunosuppressive therapies for non-oncologic conditions.

For those with CHB, exposure to a majority of immunosuppressive therapies leads to a moderate (1–10%) to high (>10%) risk of HBV reactivation. For these patients at moderate and high risk of HBV reactivation, the prophylactic NA strategy should be considered. Data from randomized control and prospective studies have demonstrated that prophylactic NA therapy can dramatically reduce, up to five-fold, risks of HBV reactivation and HBV reactivation-associated hepatitis [35–37]. For patients at low risk of reactivation, serial monitoring with on-demand NA therapy is recommended.

For patients with latent HBV, prophylactic NA therapy is recommended for those at high risk for reactivation, including patients with non-Hodgkin's lymphoma who are exposed to rituximab or similar B-cell-depleting agents. Either strategy of NA prophylaxis or monitoring with on-demand NA therapy can be employed for patients at moderate risk (1–10%), with the choice of either strategy tailored to the patient's comorbidities, history of adherence, and intended duration of immunosuppressive therapy. Patients with latent HBV and at low risk (<1%) of reactivation should undergo serial monitoring with on-demand NA treatment if there is evidence for reactivation. In situations where an on-demand strategy is limited by the unavailability or prohibitive cost of repeated HBV DNA testing, a prophylactic approach with upfront therapy is advisable.

An important monitoring and treatment principle to take note of is the potential changes in patient immunosuppressive regimens, which frequently occur due to changes in the underlying disease. For example, patients with a low-risk treatment may have progression of the primary disease, prompting an increase in their steroid doses, changes to a high-risk category therapy, and/or a combination of therapies that would now place the patients at high risk for HBV reactivation for which prophylactic antiviral would become indicated.

Finally, if prophylactic antiviral treatment is initiated, medications with high potency and high genetic barrier first-line NA therapy, such as entecavir (ETV), tenofovir disproxil (TDF), or tenofovir alafenamide (TAF), should be used. It is also imperative to continue prophylactic antiviral treatment until at least 6–12 months after the end of the immunosuppressive medications, except for those with B-cell-depleting biologics when a longer course (18 months) may be prudent due to reports of delayed to very delayed reactivation with these agents.

6. Treatment of Reactivation

All patients with HBV reactivation should be treated with first-line NA therapy, such as ETV, TDF, or TAF. These agents are preferred over older ones, such as lamivudine, telbivudine, and adefovir, since there is an increased risk of developing a drug-resistant virus with these agents. For those early in their HBV reactivation, the goal is to prevent progression towards severe hepatitis and/or hepatic failure, which can occur in up to 40% of patients who experience HBV reactivation [13]. The decision to interrupt immunosuppressive therapy should be individualized based on the patient's clinical presentation. Patients with a mild, isolated elevation in HBV DNA levels may be monitored on continued immunosuppression, particularly if NA therapy is initiated promptly. However, those with reactivation-associated hepatitis may require temporary reduction or cessation of immunosuppressive therapy until there is adequate suppression of their HBV viral load and a significant decrease in their liver enzymes.

7. HBV in Solid Organ Transplant Recipients

As with non-transplant settings, patients with CHB and latent HBV who are solid organ transplant recipients may be at risk for HBV reactivation due to the lifelong immunosuppression (e.g., corticosteroids, lymphoid-depleting agents, calcineurin inhibitors) required to prevent allograft rejection.

In addition to recipient-related HBV reactivation, there is an added consideration of donor-derived HBV infections. As of 1 March 2021 per U.S. organ procurement organization policy, all potential donors are tested with HBsAg, anti-HBc, and HBV DNA to identify those with CHB or latent HBV. For HBV-negative recipients, the use of these allografts from HBsAg-positive and/or anti-HBc-positive donors confers risk of de novo HBV infection to the recipient, requiring tailored recipient–donor matching, prophylactic strategies, and post-transplant monitoring. This section will focus on screening, risk stratification, and management strategies for HBV in solid organ transplant (SOT) recipients. For a discussion of HBV reactivation in hematopoietic stem cell transplant recipients, we direct the readers to these other reviews [38–40].

Post-transplant HBV serologic monitoring for SOT recipients deserves special attention. As the natural course of HBV infection is influenced by both viral replication and host immunity, HBV control in the post-transplant setting is heavily influenced by the degree of immunosuppression. As with the risk of post-transplant infection with Epstein–Barr virus and other opportunistic pathogens, the risk of HBV recurrence or reactivation escalates with higher degrees of immunosuppression [41]. Therefore, patients should be monitored more closely during periods of more intense immunosuppression (i.e., the first year post-transplant) with laboratory studies that include HBsAg and HBV viral load. If NA therapy fails to fully suppress the HBV DNA level, further evaluation with HBV DNA polymerase testing should be performed to guide tailored adjustments to the treatment regimen. Additional strategies to achieve complete viral suppression should be considered if not achieved with a single agent. These strategies may include combination therapy with two NAs, such as tenofovir/emtricitabine or tenofovir/entecavir. The tenofovir/emtricitabine combination offers a lower-cost, single-pill option, while tenofovir/entecavir may provide greater efficacy by combining two first-line agents.

7.1. Solid Organ Transplantation in Recipients with CHB

Prophylactic treatment should be used to prevent HBV infection of the liver allograft and post-OLT recurrent infection in the HBsAg-positive recipient/HBV-negative donor (Table 2).

Table 2. HBV recurrence or de novo infection risk according to recipient and donor HBV profile for liver transplant recipients.

Donor HBV Status	Recipient HBV Status	Suggested Management	Rationale for Management
HBV negative			
	HBsAg positive	- High-barrier NA - HBIG if high risk for reactivation, including detectable HBV DNA at transplant, NA resistance, non-adherence, co-infection with HIV and/or HDV	Highly effective in preventing HBV recurrence in transplanted allograft from extrahepatic viral reservoirs
	Anti-HBs negative/anti-HBc positive	No prophylaxis needed; on-demand NA	Low risk for recurrence as the native allograft is replaced
HBsAg negative, anti-HBc positive			
	Prior infection with immunity HBsAg negative, anti-HBs positive, anti-HBc positive	No prophylaxis needed; on-demand NA	Low risk for de novo HBV infection, estimated risk ~1% without prophylaxis
	HBV naïve, immunity via vaccination HBsAg negative, anti-HBs positive, anti-HBc negative	High-barrier NA	Elevated risk for de novo HBV infection, estimated risk ~13% without prophylaxis
	Prior infection without immunity HBsAg negative, anti-HBs negative, anti-HBc positive	High-barrier NA	Elevated risk for de novo HBV infection, estimated risk ~10% without prophylaxis
	HBV naïve and without immunity HBsAg negative, anti-HBs positive, anti-HBc negative	High-barrier NA	Very high risk for de novo HBV HBV infection, estimated risk ~47% without prophylaxis
HBsAg positive or HBV DNA positive			
	Any HBV status	High-barrier NA	NA therapy is indicated for reactivation and HBV-related complications as a transplant allograft with CHB infection

HBIG: HBV immune globulin; HIV: human immunodeficiency virus; HDV: hepatitis D virus; NA: nucleos(t)ide analog; CHB: chronic hepatitis B.

For patients with end-stage liver disease and/or hepatocellular carcinoma due to CHB, an orthotopic liver transplant can be a life-saving intervention. During the transplant surgery, the recipient's HBV-infected liver is replaced. Yet, there is a risk of HBV recurrence in the transplanted liver allograft due to extrahepatic viral reservoirs in the recipient's circulation and lymphatic system. Without any prophylaxis, the HBV recurrence rate of HBV has exceeded 80%, resulting in a poor graft and recipient survival rate of only ~50% at 5 years post-OLT [42,43].

However, these outcomes are now of historical significance due to advancements in prophylactic strategies. For OLT recipients with CHB, a combination of NA and HBIG therapy has been proven effective in reducing HBV recurrence to <5% while ensuring excellent graft and recipient survival rates [44]. Nonetheless, maintaining long-term viral suppression is crucial, underscoring the importance of selecting NAs with a high barrier to resistance. Currently, these NAs include ETV, TDF, and TAF. When selecting between these options, it is important to consider common nonhepatic comorbidities in the post-transplant population, such as chronic renal insufficiency and poor bone health. Approximately 20% of transplant recipients develop chronic kidney disease within five years post-OLT, primarily due to calcineurin inhibitors and other nephrotoxic agents [45]. Preliminary evidence suggests that TDF use is associated with higher rates of nephrotoxicity compared to other NAs [46–48]; therefore, those with renal dysfunction may benefit from using ETV or TAF long term. Older agents such as lamivudine, adefovir, and emtricitabine are not recommended due to their high rates of drug resistance, which compromise long-term viral suppression. Higher HV viral levels at the time of transplant have been associated with an increased risk of HBV recurrence [49]. Thus, all OLT candidates with CHB should receive suppressive NA therapy, with the goal of achieving as low an HBV viral level as possible and reducing the risk of HBV recurrence post-OLT.

In addition to lifelong suppressive NA therapy, many transplant programs incorporate HBIG therapy to prevent HBV recurrence after OLT [50–52]. HBIG blocks HBV nucleocapsid entry into hepatocytes and neutralizes circulating HBV via a recipient antibody-mediated immune response. HBIG protocols differ in dose and duration according to the transplant center. Recipients at low risk for recurrence receive HBIG peri-operatively or within the first week post-OLT. However, recipients with risk factors for HBV recurrence (e.g., elevated pre-transplant HBV viral load, history of non-adherence, and baseline NA antiviral resistance) are typically recommended to receive HBIG infusions up until the first year post-OLT. Those who are co-infected with HDV are also recommended an extended course of HBIG, as HBV recurrence invariably leads to HDV co-recurrence, for which there are limited therapies. However, not all programs utilize HBIG due to the added costs and unclear benefits beyond NA monotherapy alone [44,52,53].

Prophylactic treatment is used to prevent HBV reactivation in HBsAg-positive recipients of nonhepatic solid organs.

For nonhepatic (e.g., kidney, pancreas, heart, lung, etc.) solid transplant recipients with CHB, management focuses on preventing HBV reactivation, as the native HBV-infected liver remains intact (Table 3). Much of the data from nonhepatic SOT recipients are derived from kidney transplant recipients. In renal transplant recipients with CHB, the risk of HBV reactivation without antiviral prophylaxis is markedly elevated, ranging from 50% to 94%, which leads to much poorer recipient survival rates. Thus, to prevent recurrent HBV and its attendant complications, current guidelines recommend lifelong prophylactic antiviral therapy with a high-barrier NA to prevent reactivation and its associated complications. While LAM has historically improved survival in renal transplant recipients, its high rates of resistance limit its long-term use. Thus, high-barrier NAs are preferred due to their

long-term efficacy. As with OLT recipients transplanted for CHB, ETV and TAF may be preferred over TDF due to a lower risk of renal toxicity [47,48].

Table 3. HBV recurrence or de novo infection risk according to the recipient and donor HBV profile for nonhepatic solid organ transplant recipients.

Donor HBV Status	Recipient HBV Status	Suggested Management	Rationale
HBV negative			
	HBsAg positive	High-barrier NA	Prevent HBV reactivation in the setting of immunosuppression
	Anti-HBs negative/anti-HBc positive	High-barrier NA	Prevent HBV reactivation in the setting of immunosuppression
HBsAg negative, anti-HBc positive			
	HBV immune Anti-HBs positive Anti-HBc any status	No prophylaxis needed; on-demand NA	Low risk of de novo HBV infection (<1%)
	HBV non-immune Anti-HBs negative Anti-HBc any status	High-barrier NA	Prevent de novo HBV infection
HBsAg positive or HBV DNA positive			
	HBV immune Anti-HBs positive Anti-HBc any status	High-barrier NA	Prevent de novo HBV infection
	HBV non-immune Anti-HBs negative Anti-HBc any status	High-barrier NA + HBIG	Prevent de novo HBV infection

HBIG: HBV immune globulin; NA: nucleos(t)ide analog.

Prior to transplant, all SOT candidates with CHB should be evaluated by a provider with expertise in the management of HBV, and NA therapy should be initiated as per guidelines. If NA therapy is not initiated pre-transplant, it should be started at the time of transplant and continued indefinitely, as the risk of HBV reactivation persists as long as immunosuppressive therapy is required. An exception is if immunosuppression is discontinued following graft failure, such as in the case of kidney allograft failure necessitating a return to dialysis.

7.2. Solid Organ Transplantation in Recipients with Latent (Anti-HBc-Positive) HBV

OLT recipients with latent HBV (HBsAg negative, anti-HBc positive)

Recipients with latent HBV and who receive an HBV-negative liver allograft typically do not need antiviral prophylaxis due to a minimal risk of HBV recurrence [54,55]. The native liver, the main source of HBV recurrence, is removed. These patients undergo HBV viral studies (HBsAg, HBV viral load) every 1–3 months for the first year and then annually afterward.

Nonhepatic SOT recipients with latent HBV (HBsAg negative, anti-HBc positive)

In contrast to OLT recipients, nonhepatic SOT recipients retain their native liver and thus have a low, albeit substantial (<5%) risk of HBV reactivation. A systematic

review and meta-analysis of 16 retrospective cohort studies and 2913 nonhepatic SOT recipients reported an overall HBV reactivation rate of 2.5% [56]. On subgroup analyses, the reactivation rate was significantly higher in patients who were non-immune (anti-HBs negative; 7.8%) and received lymphoid-depleting therapies (7.3% for recipients who received rituximab, 4.9% for those who received anti-thymocyte globulin (ATG)). Among those with HBV reactivation, complications were frequent and serious; 11% of recipients with HBV reactivation experienced HBV-related graft failure and/or died.

Data regarding the optimal management strategy in this population remain limited. While guidelines generally do not recommend routine prophylactic antiviral therapy [50, 54,55], some centers opt for initiating NA prophylaxis in patients with higher-risk profiles, such as those who are anti-HBs negative (non-immune) and/or receiving B-cell depleting therapies, such as rituximab or other lymphodepleting agents, such as alemtuzumab or ATG. In one retrospective cohort study of 180 nonhepatic SOT recipients transplanted at the Mayo Clinic, 77 recipients received prophylactic NA therapy, and 103 recipients did not receive NA prophylaxis [57]. No recipient who received prophylactic NA therapy experienced HBV reactivation. In contrast, 12 of 97 (12%) of those who did not receive prophylaxis experienced HBV reactivation. HBV reactivation occurred in 29% (2/7) of recipients exposed to rituximab and 100% (2/2) who were exposed to lymphoid-depleting agents, such as ATG or alemtuzumab. Thus, given the excellent side effect profile of first-line antiviral agents, such as ETV and TAF, and the availability of low-cost generic ETV, it may be prudent to consider prophylactic antiviral treatment in this population.

7.3. Solid Organ Transplantation in HBV-Negative Recipients with Anti-HBc-Positive Allografts: Risk of Graft-Related de Novo HBV Infection

OLT recipients of anti-HBc-positive allografts

The availability of anti-HBc-positive allografts has significantly expanded the donor pool, particularly in HBV-endemic regions, such as Asia and Africa, without compromising recipient or graft survival. While these allografts may be best allocated to recipients with CHB (i.e., recipient HBsAg positive), with the appropriate prophylactic strategies, even HBV-negative (i.e., HBsAg negative) recipients of an anti-HBc-positive liver can achieve excellent outcomes. A retrospective study from Hong Kong described a similar 10-year graft survival rate for 416 recipients of anti-HBc-positive grafts (76.8%) when compared to 548 recipients of anti-HBc-negative grafts (74.8%) and without any difference in graft dysfunction, recipient death, or hepatocellular carcinoma [58].

However, the utilization of these liver allografts does carry a risk of de novo HBV infection to the recipient via transmission from the transplant allograft. The risk depends also on the recipient's HBV serologies. Without any prophylaxis, recipients who are HBV naïve/non-immune (anti-HBc negative, anti-HBs negative) are at the highest risk for de novo HBV infection (~48%), followed by recipients who are HBV exposed/non-immune (anti-HBc positive, HBsAg negative, anti-HBs negative; risk~13%) and then recipients who are vaccinated (anti-HBc negative, anti-HBs positive; HBV risk~9%) [59]. Recipients who are naturally immune (anti-HBc positive, anti-HBs positive; HBV risk ~1%) have the lowest risk of de novo HBV infection.

Many transplant programs administer prophylactic NA to recipients at higher risk of de novo HBV infection (e.g., all recipients except those naturally immune). In a meta-analysis of 26 studies and 462 recipients of anti-HBc-positive liver allografts, NA prophylaxis substantially reduced the risk of de novo HBV infection from 58% to 11% in HBV-naïve/non-immune (anti-HBc negative, anti-HBs negative), 18% to 2% in vaccinated (anti-HBc negative, anti-HBs positive), and 14% to 3% in HBV-exposed/non-immune recipients (anti-HBc positive, anti-HBs negative) [60]. In this study, NA prophylaxis did not

reduce the risk of de novo hepatitis in recipients who were naturally immune (anti-HBc positive, anti-HBs positive). HBIG is not typically utilized as it does not provide additional benefits for preventing de novo HBV infection beyond NA monotherapy in this setting [59].

Nonhepatic SOT recipients of anti-HBc-positive allografts

The risk of transmission for anti-HBc-positive allografts is mainly observed in OLT recipients; nonhepatic SOT recipients have much lower risks of de novo HBV infection (<1%). In a recently published meta-analysis of 13 studies and 2516 recipients of anti-HBc-positive kidney allografts, only nine (0.36%) cases were reported [61]. Notably, the risk of HBV infection was significantly higher among recipients who did not receive prophylaxis and lacked immunity, with a rate of 5.71% (2/35) observed in those who were anti-HBc positive and anti-HBs negative. There were no differences in recipient or graft survival for recipients of anti-HBc-positive vs. anti-HBc-negative kidney allografts. Similarly low rates of HBV infection and excellent outcomes have been reported for the use of anti-HBc-positive thoracic allografts, although data are scarce [62–64].

All potential SOT recipients who are HBV non-immune should be vaccinated. The optimal prophylactic strategies for recipients of nonhepatic anti-HBc-positive allografts post-transplant remain uncertain, but based on limited data regarding a higher risk of infection for non-immune patients [61], NA prophylaxis can be considered for those who are HBV non-immune. In the absence of NA prophylaxis, routine post-transplant monitoring, including HBsAg, HBV DNA every one to three months, and on-demand therapy, is recommended.

7.4. Solid Organ Transplantation in HBV-Negative Recipients with HBsAg-Positive Allografts: Acquired Chronic HBV Infection

OLT recipients of HBsAg-positive allografts

Liver allografts from donors who are HBsAg positive or HBV nucleic acid testing (NAT) positive are not routinely utilized, as their use typically results in chronic HBV infection in recipients, which invariably results in chronic HBV infection. However, a growing body of literature suggests that HBsAg-positive allografts can effectively expand the donor pool and achieve similar patient and graft outcomes to those of HBsAg-negative ones [65–69]. Ali et al. compared clinical outcomes in 209 OLT recipients of HBV-positive allografts (defined as HBsAg positive or HBV NAT positive) to 1045 matched recipients of HBV-negative allografts using data from the Organ Procurement and Transplantation Network (OPTN) database. This study found no statistically significant differences in recipient mortality (3-year survival: 84.8% for the HBV-positive group vs. 82.3% for the HBV-negative group, $p = 0.47$) or graft loss (3-year graft survival: 77.9% for the HBV-positive group vs. 79.7% for the HBV-negative group, $p = 0.72$). Similar findings were reported using data from the China Liver Transplant Registry [66]. Among 259 recipients of HBsAg-positive allografts and 259 matched recipients of HBsAg-negative allografts, the investigators observed comparable 3-year survival rates (60.4% vs. 69.1%, respectively, $p = 0.062$). These studies highlight the potential for HBV-positive allografts to safely increase the available donor pool without compromising outcomes. Prior to transplant, a thorough histologic evaluation of the donor's liver was performed to confirm the absence of significant fibrosis. Additionally, grafts from donors with known HDV co-infection should be discarded given the lack of effective therapies for chronic HDV infection [70].

Transplantation with these HBV-positive liver allografts almost invariably results in chronic HBV infection in the recipient, making long-term suppression with NA therapy essential. High barrier-to-resistance NAs, such as ETV, TDF, and TAF, should be prioritized. If the recipient does not respond adequately to first-line NA therapy, additional strategies to achieve complete viral suppression should be explored, including HBV DNA polymerase

sequencing, to identify resistance mutations and tailor therapy accordingly. The role of HBIG is likely limited in recipients of HBsAg-positive liver allografts, as HBIG only prevents HBV infection of the allograft but does not provide any therapeutic benefits once HBV infection has already occurred, as is the case with HBV-positive donor allograft transplant.

Another important consideration in the recipient of an HBsAg-positive graft with resultant chronic hepatitis B infection is the risk of de novo HCC in these immunosuppressed individuals. Given that HBV is a pro-oncogenic pathogen, there is concern for an increased risk of HCC in recipients of HBV-positive allografts. While the absolute risk of HCC in this transplant population is not well defined, adequate viral suppression with NAs has consistently been shown to significantly reduce the risk of HCC in the immunocompetent population [71,72]. Given the increased risk of HCC due to the use of immunosuppressants in a post-OLT population, it is important to consider HCC screening for all recipients of HBV-positive allografts with liver imaging (e.g., ultrasound, computed tomography, or magnetic resonance imaging) and alpha-fetoprotein (AFP) every six months. This is particularly critical for recipients of grafts from older donors of Asian or African descent, who may carry an elevated baseline risk for HCC.

Nonhepatic transplant recipients of HBsAg-positive allografts

The use of nonhepatic organs from HBsAg-positive donors has historically been considered marginal due to the risk of HBV transmission. However, nonhepatic allografts can also be utilized to improve the donor pool and transplant access [73,74]. While the liver is the main source of HBV transmission, HBV remains present in the circulation and lymphocytes, presenting a lower but still notable risk of de novo HBV infection [75].

Preliminary data have demonstrated satisfactory outcomes when recipients are treated with antiviral therapy, with or without HBIG prophylaxis. Delman et al. found that among fifty-six kidney transplant recipients of HBV NAT-positive donors, nine (16.7%) developed de novo HBV infection and active viremia [67]. All nine patients achieved HBsAg clearance after NA therapy with ETV and none developed any HBV-related complications. Graft and recipient survival rates were excellent, suggesting that effective antiviral therapy can mitigate the risks associated with HBV-positive allografts. Tuncer et al. observed no cases of de novo HBV infection among 35 HBV-immune recipients of kidneys from HBsAg-positive and HBV DNA-negative living donors [76]. Of interest, neither HBIG nor NA was used prophylactically for the patients in this study. These findings suggest that natural HBV immunity may mitigate the risk of DNH in such cases. Finally, Yin et al. reported on 105 recipients of kidneys from HBsAg-positive, HBeAg-positive, and HBV DNA-positive donors [74]. Outcomes were compared with those of recipients of kidneys from HBsAg-negative/anti-HBc-positive donors, and graft and survival rates were found to be similar. Four (3.8%) recipients developed an HBsAg-positive de novo infection; all four patients had subsequent conversion to HBsAg-negative status following NA therapy. There is limited data on thoracic organ transplantation involving HBsAg-positive donors, but satisfactory outcomes have also been reported. In a meta-analysis by Yost et al. of heart transplant recipients, 1 of 11 recipients of HBsAg-positive donors developed de novo HBV infection post-transplant, which was managed with lamivudine.

The optimal management of recipients of HBsAg-positive donors is unknown, but the risk of de novo HBV infection likely exceeds that of nonhepatic, SOT recipients of anti-HBc-positive allografts, and the prophylactic strategy should be tailored accordingly [54,55]. Prophylactic NA therapy (combined with HBIG therapy for non-immune recipients) should be considered for all nonhepatic SOT recipients. Routine laboratory monitoring should include assessments of HBsAg and HBV DNA, and liver function tests should be considered at least every three months to detect any signs of HBV transmission.

8. Conclusions

In conclusion, effective management of HBV reactivation and donor-related HBV transmission in immunosuppressed and solid organ transplant populations hinges upon a risk-stratified approach tailored to HBV serostatus, the type of immunosuppression, and patient-specific factors, including HBV status of donors and patient ability to adhere to long-term monitoring, especially if not prophylactically treated. The use of high-barrier NA therapies such as ETV, TDF, or TAF has proven instrumental in mitigating the risks of HBV reactivation and attendant complications in immunosuppressed patients from both non-transplant and transplant settings. For transplant recipients, the use of HBV-positive allografts, while historically limited, has emerged as a viable strategy to expand the donor pool, achieving recipient and graft survival rates comparable to those of HBV-negative allografts when appropriate prophylaxis is employed. The use of these HBsAg-positive allografts merits further investigation, including an assessment of the optimal prophylactic strategies and the long-term risk of HCC development.

Author Contributions: Conceptualization, P.V. and M.H.N.; methodology, P.V. and M.H.N.; writing—original draft preparation, P.V. and M.H.N.; writing—review and editing, P.V. and M.H.N.; visualization, P.V. and M.H.N.; supervision, M.H.N. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Conflicts of Interest: The authors declare no conflict of interest.

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viruses

MDPI

Review

Herpesvirus Infections After Chimeric Antigen Receptor T-Cell Therapy and Bispecific Antibodies: A Review

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Abstract: In this narrative review, we explore the burden and risk factors of various herpesvirus infections in patients receiving chimeric antigen receptor T-cell (CAR-T) therapy or bispecific antibodies (BsAb) for the treatment of hematologic malignancies. Antiviral prophylaxis for herpes simplex/varicella zoster viruses became part of the standard of care in this patient population. Breakthrough infections may rarely occur, and the optimal duration of prophylaxis as well as the timing of recombinant zoster immunization remain to be explored. Clinically significant cytomegalovirus (CMV) infections can affect up to 10% of patients after CAR-T, depending on the CAR-T product target, post-CAR-T complications such as cytokine release syndrome and the need for glucocorticoid therapy. Surveillance and prophylactic strategies for CMV need to be developed, whereas the risk factors for and the burden of CMV infections after BsAb are not yet well-defined. Human herpes virus 6 reactivation and end organ disease such as encephalitis are rarely reported after CAR-T and have not yet been reported after BsAb; additional research is needed.

Keywords: herpesviruses; CAR T cell therapy; bispecific antibodies; HSV; VZV; CMV; HHV-6

1. Introduction

Herpesvirus infections represent a significant infectious complication after hematopoietic cell transplantation (HCT) and can significantly affect patients with hematologic malignancies [1]. As new therapeutic modalities for hematologic malignancies emerge, such as chimeric antigen receptor T-cell (CAR-T) therapy or bispecific antibodies (BsAb), our understanding of the burden and risk factors for herpesvirus reactivations in patients receiving these novel therapies remains limited. This narrative review explores the available data on the incidence and risk factors for herpes simplex virus (HSV), varicella zoster virus (VZV), cytomegalovirus (CMV) and human herpesvirus 6 (HHV-6) in patients receiving currently approved CAR-T therapy or BsAb for the treatment of hematologic malignancies, reviewing current recommendations on prevention, management and monitoring and offering directions for future research.

2. Herpesviruses and Chimeric Antigen Receptor T-Cell (CAR-T) Therapy

Herpesvirus reactivation has been reported since the early clinical trials of CD19 CAR-T in patients with B-ALL and B-cell lymphomas. One of the first studies of infectious complications of CD19 CAR-T by Hill et al. evaluated 133 patients enrolled in a phase

1/2 study of CD19 CAR-T, all of whom received acyclovir or valacyclovir for HSV/VZV prophylaxis, and one patient had CMV reactivation without end organ disease in the first 28 days after CAR-T [2]. Between day 29 and 90 post CAR-T, one patient developed CMV reactivation, and another one developed CMV pneumonitis, neither of whom had a history of HCT [2]. Additional retrospective studies seemed to reflect a low incidence of herpesvirus reactivation after CD19 CAR-T, with occasional cases of HSV/VZV, most of whom were not on acyclovir prophylaxis, and a few cases of CMV DNAemia [3–5]. Nevertheless, a study of 41 patients with large B-cell lymphoma suggested a more significant burden of herpesviruses, affecting a quarter of their cohort, with one case of HHV-6 meningoencephalitis in the first 28 days, four cases of CMV reactivation within two weeks of glucocorticoid initiation, and six cases of herpes zoster occurring beyond 28 days post CD19 CAR-T [6].

The above findings led to the recognition of herpesvirus reactivation as a potential infectious complication of CD19 CAR-T and, by extension, of B-Cell Maturation Antigen (BCMA) CAR-T, where the data are more limited. In addition to the on-target, off-tumor effects of both CD19 and BCMA CAR-T causing B-cell/plasma cell aplasia and subsequent hypogammaglobulinemia [7], significant impairment in T-cell-mediated immunity occurs. Administration of lymphodepletion chemotherapy, typically with fludarabine and cyclophosphamide, precedes the infusion of CAR-T [8]. The nature of the lymphodepleting chemotherapy regimen, particularly the dose of cyclophosphamide, affects infection risk [3]. Additionally, a significant proportion of patients develop cytokine release syndrome (CRS) and immune-effector cell-associated neurotoxicity syndrome (ICANS) after CAR-T, the treatment of which requires further immunosuppression, with tocilizumab, anakinra and glucocorticoids [8]. Furthermore, many patients, particularly early after CAR-T approval, received the latter after failing multiple lines of therapy, including autologous and allogeneic HCT (36–55% of patients in some of the aforementioned cohorts [2–4]); thus, these patients entered the CAR-T stage of their treatment with an already unfavorable net state of immunosuppression. While this might not be the case for some CD19 CAR-T recipients as those products are used earlier as lines of treatment, this remains an important factor for recipients of BCMA CAR-T. These factors may lead to a slow recovery of T-cell-mediated immunity after CAR-T, as demonstrated by prolonged CD4 lymphopenia [9], an established pathophysiological mechanism and immunological indicator of risk for herpesvirus reactivations after allogeneic HCT [1]. A study of 31 patients who received CD19 CAR-T for B-cell lymphoma under clinical trial showed delayed CD4 recovery in three out of nine patients tested one year after CAR-T, and in two out of seven patients tested two years after CAR-T [9]. A large retrospective study evaluated 160 patients who received FDA-approved CD19 CAR-T and identified grade 3/4 CRS, grade 3/4 ICANS, a higher cumulative dose of glucocorticoids in the first 30 days, and administration of anakinra as independent risk factors for herpesvirus reactivation [10]. Additionally, a large database analysis, including 2256 patients who received CD19 or BCMA CAR-T, reported a 13.6% prevalence of herpesvirus reactivations, occurring at a median of 71 days post CAR-T (IQR 18–252 days), with CMV being the most common (7.5% of patients) followed by other herpesviruses (<3% of patients each) [11]. Independent risk factors for herpesvirus reactivations in this study included prior HCT, HIV, hypogammaglobulinemia, ICANS, hemophagocytic lymphohistiocytosis, rituximab and anakinra [11]. Patients with diffuse large B-cell lymphoma or mantle cell lymphoma were associated with a lower risk of herpesvirus reactivations [11].

In this section, we will review specific studies regarding HSV/VZV, CMV and HHV-6 after CAR-T. We excluded EBV as the clinical significance of EBV detection after CAR-T in patients with B-cell malignancies remains to be determined. It is worthwhile noting that

CD19 CAR-T products have been used in the treatment of post-transplant lymphoproliferative disease (PTLD) [12], but no cases of PTLD occurring after autologous CAR-T have been reported so far.

2.1. HSV/VZV

The risk of HSV and VZV reactivations after CAR-T is widely acknowledged, and many institutions implemented antiviral prophylaxis as standard of care, which is reflected in the high rates of antiviral prophylaxis in published clinical trials and real-world data [2,4,5,10]. The majority of reported cases of HSV and VZV reactivations after CAR-T occurred in patients who were not on antiviral prophylaxis in the earliest clinical trials [3,4,9]. In a cohort of 31 patients who received CD19 CAR-T under a clinical trial without antiviral prophylaxis, five events of VZV reactivation and three events of HSV reactivation were reported [9]. Hence, most institutions and professional societies recommend antiviral prophylaxis, including the American Society of Transplant and Cellular Therapy (ASTCT) which recommends acyclovir or valacyclovir from the initiation of lymphodepletion until at least 6 months post CAR-T [13]. Although prophylaxis is common, breakthrough infections have been rarely reported [5], including severe complications such as VZV retinitis [14] and HSV pneumonia [15]. As our understanding of the immune reconstitution after CAR-T evolves, the optimal duration of antiviral prophylaxis remains to be fully determined and the role of CD4 count monitoring as a surrogate marker of immune reconstitution may warrant additional studies.

There are limited data on the serologic response to the recombinant zoster vaccine after CAR-T. While a diminished antibody response is anticipated after both CD19 and BCMA CAR-T due to on-target, off-tumor effects on B lymphocytes and plasma cells, the pathogen-specific antibody response is more significantly impaired after BCMA CAR-T compared to CD19 CAR-T, likely due to the impact of BCMA CAR-T on antibody-producing plasma cells [7]. The pathogen-specific antibody response is likely preserved after CD19 CAR-T, especially in the absence of prior allogeneic HCT [16]. A prospective, cross-sectional study that evaluated vaccine-preventable diseases including VZV showed that CD19 CAR-T recipients had IgG levels correlating with seroprotection comparable to the general population, whereas BCMA CAR-T recipients were about 50% less likely to achieve these IgG levels, with fewer pathogen-specific epitope hits compared to CD19 CAR-T recipients [17]. The ASTCT recommends the recombinant zoster vaccine (Shingrix®) for VZV-seropositive adult CAR-T recipients or those with prior varicella or zoster infections [13], although the efficacy and the right schedules still need to be determined. The proposed schedule based on the guidelines suggested that the recommended first and second doses be administered at least 12 and 18 months post-CAR-T, provided the patient is >1 year post HCT, >8 months off systemic immunosuppressive therapy and with an absolute CD4 count > 200 cells/μL [13]. This immunization schedule in relation with the timing of antiviral prophylaxis discontinuation needs further investigations.

2.2. CMV

CAR-T recipients vary in terms of baseline risk for CMV prior to infusion. While those who receive CAR-T for B-ALL are more likely to have had prior allogeneic HCT and thus be at higher risk for CMV [1], the latter has not been reported as a common or frequent infectious complication of lymphoma or multiple myeloma therapy prior to the CAR-T era. In a prior study by our group at a large comprehensive cancer center over a 4-year period, only 84 patients with lymphoma or multiple myeloma on different lines of therapy developed clinically significant CMV infection (CS-CMVi is defined as CMV end organ disease and/or CMV DNAemia leading to preemptive antiviral therapy based on

prespecified thresholds [18,19]. Nevertheless, CS-CMVi carried). Nevertheless, CS-CMVi carried significant morbidity and mortality in this cohort, as 63% of the patients were diagnosed with CMV end organ disease, 19% had recurrent CS-CMVi and 7% had CMV-attributable mortality [20]. The high proportion of CMV end organ disease could be partly explained by the lack of prospective monitoring for CMV in the plasma or blood, with subsequent delay in antiviral therapy that could have prevented progression to pneumonia, in particular. Within this context, earlier studies in CAR-T recipients reported low rates of CMV reactivation with almost no CMV end organ disease [2–5], although few case reports described CMV end organ disease such as pneumonia [15,21] and retinitis in this patient population [22,23]. These clinical observations prompted further studies of CMV after CAR-T, most of which are retrospective and are limited by a heterogeneity in CMV surveillance protocols and the CMV viral load thresholds to initiate preemptive antiviral therapy [24–29] (Table 1).

Table 1. Studies of CMV reactivation after CAR-T.

CAR-T Product	Number of Patients	CMV Surveillance Protocol	Duration of Follow-Up	% with Any CMV Reactivation	% with CS-CMVi	% with CMV Disease	Risk Factors for CMV	Reference
CD19, BCMA	2256	None	Median 420 days	7.5%	N/A	N/A	Not reported	[11]
CD19	230	Weekly if neutropenia or grade 3/4 CRS/ICANS	365 days	22%	10%	3%	Asian/Middle Eastern Treatment for CRS/ICANS Meeting criteria for surveillance	[24]
CD19, BCMA	95	None	Median 352 days	33%	11%	0	2+ immunosuppressants	[25]
CD19	65	None	365 days	22%	15%	1.5%	Not reported	[26]
CD19	105	Weekly × 4 weeks	Minimum 30 days	44%	3%	0	Dexamethasone	[27]
CD19	51	Days 0, 7, 14, 21, 30, 60 and 90	90 days	56%	6%	0	Axicabtagene cileucel	[28]
CD19	60	Once at 14–21 days	30 days	17%	10%	0	Not reported	[29]
CD19, CD20, BCMA	72	Weekly × 12 weeks	12 weeks	27%	7%	0	Glucocorticoids > 3 days BCMA CAR-T	[30]

Abbreviations—CMV, cytomegalovirus; CAR-T, chimeric antigen receptor T-cell therapy; CS-CMVi, clinically significant CMV infection; N/A, not available; CRS, cytokine release syndrome; ICANS, immune effector cell-associated neurotoxicity syndrome.

One of the largest retrospective cohort studies examined CMV infections in 230 CD19 CAR-T recipients over a 3-year period, with weekly plasma CMV PCR performed in patients with neutropenia or those with grade 3/4 CRS/ICANS for up to one month after CAR-T [24]. CMV reactivation at any level occurred in 51 (22%) patients; 22 (10%) had CS-CMVi at a median of 17 days (range 0–343 days) after CAR-T [24]. Interestingly, 7 out of these 22 (33%) patients developed CMV end organ disease [24]. Independent risk factors for CS-CMVi included Asian or Middle Eastern ethnicity (HR 13.71), treatment of CRS or ICANS with steroids (HR 6.25) and being monitored for CMV reactivation by PCR (HR 6.91) [24]. Similar findings in terms of prevalence and burden of CMV infections after

CAR-T were reported in other studies [11,25–29] and are summarized in Table 1. Overall, CMV reactivations happen early, at a median of 14–21 days [25,27–29], in up to half of the patients; however, CS-CMVi was significantly less common, with prevalence ranging between 3% and 15% (Table 1). Risk factors for any CMV reactivation included two or more immunosuppressants [25], dexamethasone [27] or the use of axicabtagene cileucel (attributed to a higher rate of CRS with this product) [28]. In a recent large database analysis of viral infections after CAR-T, the prevalence of CMV reactivation was 7.5%, occurring at a median of 60 days (IQR 20–215), earlier than all other viral infections reported in this study, and with a median CMV viral load of 1719 IU/mL (IQR 808–7500 IU/mL) [11]. In a prospective study of 72 CMV-seropositive adults who received CD19, CD20 or BCMA-CAR-T and had surveillance with plasma CMV PCR at baseline and weekly up to 12 weeks post CAR-T, the cumulative incidence of CMV reactivation was 27% by week 12, with a median CMV viral load of 127 IU/mL, and most reactivations occurring between 2 and 6 weeks after CAR-T [30]. While only seven (10%) patients met the institutional threshold for pre-emptive therapy, five (7%) were treated [30]. Glucocorticoid use for >3 days and the use of BCMA CAR-T were significantly associated with a higher risk of CMV reactivation. BCMA CAR-T recipients had a higher number of prior lines of therapy, including a higher rate of prior HCT, which likely increased their net state of immunosuppression [30]. Additionally, this study evaluated CMV-specific cell-mediated immunity (CMV-CMI) at baseline and weeks 2 and 4 post CAR-T and demonstrated lower CMV-CMI at week 2 compared to baseline, with recovery to baseline levels by week 4, a pattern more pronounced in patients who developed CMV reactivation [30].

Furthermore, the relationship between CMV and post-CAR-T mortality has been explored. In the large cohort of 230 patients receiving CD19 CAR-T aforementioned, CS-CMVi within 1 year post CAR-T was significantly associated with a higher risk of non-relapse mortality (OR 2.49), despite the overall low rate of CMV end organ disease (3%), highlighting the potential indirect effects of CMV in this patient population, similar to the allogeneic HCT recipients [24,31]. The association of any CMV reactivation with an increased risk of mortality was also demonstrated in two other studies [25,26]. These studies are limited by their retrospective nature, the heterogeneity of the CMV definitions used and of the CMV monitoring protocols (or lack thereof). In summary, CMV reactivations are common after CAR-T, but CS-CMVi and CMV end organ disease remain rare. Most CMV reactivations occur early on after CAR-T in the context of a dip in CMV-CMI and disproportionately affect patients with CRS/ICANS, particularly those receiving glucocorticoids, and possibly recipients of BCMA CAR-T compared to CD19 CAR-T. At this time, CMV monitoring could be considered in the first 2 to 6 weeks after CAR-T in these higher risk patients [13]. While CMV reactivations have been associated with a higher risk of mortality, including non-relapse mortality, in CAR-T recipients, a direct causal relationship is still not established, particularly given the low rates of CMV end organ disease. Adequately powered prospective studies with systematic CMV monitoring are necessary to definitively assess the impact of CMV on overall and non-relapse mortality after CAR-T. Whether pre-emptive or prophylactic antiviral therapy (with letermovir for example) in this patient population may alter outcomes such as CMV end organ disease or all-cause mortality, as it does in allogeneic HCT recipients [32,33], needs to be determined in future trials.

2.3. HHV-6

Several cases of encephalitis secondary to HHV-6 after CAR-T have been reported [26,34–36], as well as a case of fatal HHV-6 myelitis with ascending flaccid paralysis and neuromuscular respiratory failure [37]. The diagnosis of HHV-6 encephalitis after CAR-T is challenging since the clinical presentation may overlap with ICANS [36]. Studies

on the prevalence and significance of HHV-6 reactivation and risk of encephalitis after CAR-T are sparse. In a retrospective cohort of 230 CD19 CAR-T recipients, we identified 13 (6%) patients with HHV-6 reactivation but only 1 patient with HHV-6 encephalitis, when testing for HHV-6 was at the discretion of the treating provider [38]. Similar results were reported in a prospective study of 84 CAR-T recipients (including CD19 and BCMA CAR-T) with baseline and weekly plasma HHV-6 PCR testing up to week 12 post CAR-T. The cumulative incidence of HHV-6 reactivation was 6%, with all reactivations occurring between 2 and 6 weeks post CAR-T, and with no cases of encephalitis [39]. While neither study systematically tested cerebrospinal fluid for HHV-6, the low rate of clinical encephalitis leading to testing is encouraging.

While HHV-6 encephalitis appears to be rare after CAR-T, other potential manifestations of HHV-6, such as pneumonitis and its indirect effects, remain to be determined [40]. Additionally, the clinical significance of HHV-6 DNAemia is complicated by inherited chromosomal integration, for which testing is not yet widely available, and may explain a proportion of HHV-6 detections [39]. Interestingly, CAR T cells may super express HHV-6 in patients in vivo, postulating that cellular therapy products could be the source of transmission of HHV-6 in some of these patients [41]. The extent of this phenomenon remains unknown, since primary HHV-6 infection is quite common and typically occurs during childhood [40,42]. At this time, routine monitoring for HHV-6 after CAR-T is not recommended, and testing should be guided by clinical suspicion for end organ disease, particularly in CAR-T recipients with central nervous system symptoms and no alternative diagnosis or with poor response to ICANS treatment [13,40].

3. Bispecific Antibodies

Data on herpesvirus reactivations after BsAb are scarce, due to the limited reporting of infections in the clinical trials for these agents [43,44]. Additionally, many BsAb were approved through accelerated pathways, based on phase 1/2 trials with heterogeneous patients, smaller sample sizes, and varying dosing schedules [45].

Multiple platforms of BsAb are available or in development, with the bispecific T-cell engager (BiTE) being the most widely recognized [46]. The most common T cell epitope in BsAb is CD3, while the tumor target epitope varies depending on the tumor [46]. As such, blinatumomab, the first approved BsAb for B-ALL, is an anti-CD19xCD3 BsAb [46]. An alternative tumor antigen for B-cell lymphoma is CD20 [46]. In multiple myeloma, some of the BsAb constructs target BCMA (teclistamab, erlanatamab), whereas others target non-BCMA epitopes on plasma cells such as GPRC5D (talquetamab) [47–49]. Similarly to CAR-T, BsAb therapy has been associated with CRS and neurotoxicity, although at lower rates and severity. These complications may require further immunosuppression with IL-6 blockade and glucocorticoids, in patients who have already received multiple lines of therapy prior to BsAb and can be profoundly immunosuppressed [46]. Additionally, CD19/20- and BCMA-targeting BsAb have been associated with hypogammaglobulinemia, as an on-target, off-tumor effect [46], with depletion in IgG1 and IgG3 subclasses associated with an increased risk of CMV [50,51]. Prolonged cytopenias have also been described after BsAb, as a result of their inadvertent activation of regulatory T cells [52]. Furthermore, persistent antigen exposure and continuous CD3 receptor signaling have been associated with T-cell exhaustion during BsAb therapy, which, combined with the dampening of cytotoxic T-cell function by activated regulatory T cells, can explain an increased risk of viral infections, including herpesvirus reactivations [53,54]. Treatment-free intervals can improve T-cell exhaustion related to continuous BsAb exposure [54].

On the other hand, the lack of standardized reporting of infections in clinical trials and real-world studies for BsAb remains a significant concern as it may confound or delay the appropriate diagnosis of infections and its impact on outcomes.

3.1. Blinatumomab

In a phase 3 trial comparing blinatumomab, an anti-CD19xCD3 BsAb approved for B-ALL [46], to chemotherapy in B-ALL, fifteen patients (6%) in the blinatumomab arm had oral herpes, compared to nine patients (8%) in the chemotherapy arm; one patient had grade $\geq$ 3 herpes zoster and one patient had grade $\geq$ 3 oral herpes, both in the blinatumomab arm [55]. As a result, HSV/VZV antiviral prophylaxis is recommended in patients on blinatumomab [56]. In a phase 2 single-arm trial of dasatinib with glucocorticoids followed by blinatumomab in 63 patients with B-ALL, seven patients had grade $\geq$ 2 CMV infections [57], while it is worth noting that dasatinib alone has been associated with CMV colitis [58]. Data are too limited to make a recommendation regarding CMV monitoring in these patients.

3.2. BsAb in Patients with Lymphoma

Three anti-CD20xCD3 BsAb are currently approved for relapsed/refractory B-cell lymphomas, epcoritamab, glofitamab and mosunetuzumab [45]. A meta-analysis of infections in early phase clinical trials and observational studies of BsAb for lymphoma included 2228 patients and reported nine HSV/VZV reactivations, two CMV reactivations (one of which was deemed fatal) and two EBV reactivations across all four BsAb products included [45]. A single-center retrospective study of 44 patients with relapsed/refractory non-Hodgkin's lymphoma treated with mosunetuzumab reported three episodes of herpes zoster [59].

3.3. BsAb in Patients with Multiple Myeloma

Teclistamab, an anti-BCMAxCD3 BsAb, is the first approved BsAb for the treatment of multiple myeloma, based on the phase 1/2 MajesTEC-1 trial [47,60]. A detailed analysis of infections in this trial was published 3 years after the original trial publication and included 165 patients who received teclistamab, 93% of whom received HSV/VZV prophylaxis [61]. Four (2%) patients developed oral HSV infection, three (2%) patients developed herpes zoster infection and three (2%) patients had CMV infections [54]. Erlanatamab is another anti-BCMAxCD3 BsAb approved for multiple myeloma. A total of 224 patients enrolled in two phase 1 and 2 trials for erlanatamab, and CMV infection occurred in 14 (6%) patients, including 2 who had CMV pneumonia [48,62]. Most patients in the phase 2 trial received HSV/VZV prophylaxis (87%) [48].

Talquetamab is an anti-GPRC5DxCD3 BsAb also approved for multiple myeloma, with the notable difference of targeting GPRC5D, an orphan receptor which is expressed on malignant plasma cells, unlike BCMA, which is expressed on malignant and healthy plasma cells, and mature B lymphocytes [49]. In a phase 1 trial, no CMV reactivations were reported in the 232 participants; however, 1 patient had disseminated VZV infection and 1 patient had ophthalmic herpes [49]. A pooled analysis of 1185 patients with multiple myeloma treated with BsAb monotherapy showed lower rates of neutropenia and of grade 3/4 infections in patients receiving non-BCMA BsAb compared to patients receiving BCMA-targeted BsAb [63]. This same analysis reported CMV infection and/or reactivation in 8% of the overall pooled cohort [63].

Few retrospective studies have offered additional perspectives to the early use of BsAbs in multiple myeloma (Table 2). In the majority of the studies, patients received HSV/VZV prophylaxis and, subsequently, HSV or VZV breakthrough infections were rare [64–67]. CMV reactivation at any level seemed more common, at an average of 10%

(ranging from 3% [68] to 22% [69]). On the other hand, one study reported a prevalence of 11% for CS-CMVi, including two patients with CMV esophagitis and two patients with CMV PCR > 1000 IU/mL requiring pre-emptive antiviral therapy [69].

Table 2. Studies of herpesvirus reactivations after BsAb.

BsAb Product	Number of Patients	% with Any CMV Reactivation	% with HSV/VZV	Reference
Any	39	18%	2%	[64]
Any	90	3% (No CMV disease)	-	[68]
BCMA	37	22% (11% CS-CMVi, 6% CMV disease)	-	[69]
BCMA	188	9%	0.5%	[65]
BCMA, GPRC5D	229	3.5%	1%	[66]
BCMA	55	4%	5%	[67]

Abbreviations—BsAb, bispecific antibody; CMV, cytomegalovirus; CS-CMVi, clinically significant CMV infection; HSV, herpes simplex virus; VZV, varicella zoster virus.

While the data on herpesvirus reactivations and associated risk factors after BsAbs for multiple myeloma remain largely descriptive and scarce, there is a consensus recommendation among multiple expert groups in favor of HSV/VZV prophylaxis during BsAb therapy and likely until immune reconstitution [70–72]. Even though the rates of CMV reactivation seem significant with reported cases of end organ disease, additional data are needed to determine the subset of patients at high-risk for CMV infections and who would benefit from targeted monitoring or prophylactic strategies. Finally, the burden and relevance of HHV-6 DNAemia after BsAb remain to be determined.

4. Future Directions

While antiviral prophylaxis for HSV/VZV in patients receiving CAR-T or BsAb became standard of care, a better understanding of immune recovery and vaccine response after either line of therapy is important to determine the appropriate indications and timing of recombinant zoster immunization and duration of antiviral prophylaxis in this patient population. Prospective studies to identify the subset of BsAb recipients at highest risk for CMV to develop appropriate CMV monitoring or preventive strategies in patients receiving CAR-T or BsAb and to evaluate the potential role of primary prophylaxis in the subset of high-risk patients are crucial. Finally, a better understanding of the role of HHV6 remains necessary in this patient population.

Author Contributions: Conceptualization—J.S. and R.F.C. Writing—Original Draft—J.S. and E.A.S. Writing—Review and Editing—J.S., E.A.S. and R.F.C. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Conflicts of Interest: J.S. reports research grants paid to his institution from Ansun BioPharma (San Diego, CA, USA), Community Infusion Solutions (Dallas, TX, USA), F2G (Manchester, UK), Mundipharma (Cambridge, UK), Pulmotect (Houston, TX, USA), and Shionogi (Osaka, Japan). E.A.S. reports no conflicts of interest. R.F.C. is a consultant and/or advisor for ADMA Biologics (Ramsey, NJ, USA), AiCuris (Wuppertal, Germany), Ansun BioPharma (San Diego, CA, USA), Astellas Pharma Inc. (Tokyo, Japan), Karius (Redwood City, CA, USA), Merck & Co., Inc./MSD (Rahway, NJ, USA), Moderna, Inc. (Cambridge, MA, USA), Pfizer (New York, NY, USA), Oxford Immunotec

(Abingdon, UK), Shionogi Inc. (Osaka, Japan), Eurofins-Viracor (Lenexa, KS, USA), Assembly-Bio (San Francisco, CA, USA), Tether Therapeutics (Atlanta, GA, USA), SymBio (Tokyo, Japan), and The Takeda Pharmaceutical Company Limited (Tokyo, Japan), and reports research grants paid to his institution from AiCuris (Wuppertal, Germany), Ansun BioPharma (San Diego, CA, USA), Eurofins-Viracor (Lenexa, KS, USA), Genentech, Inc. (San Francisco, CA, USA), Karius (Redwood City, CA, USA), Merck (Rahway, NJ, USA), SymBio (Tokyo, Japan), and The Takeda Pharmaceutical Company Limited (Tokyo, Japan).

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viruses

MDPI

Review

Antiviral Stewardship in Transplantation

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Abstract: Though antimicrobial stewardship programs (ASPs) are required for hospitals, the involvement of transplant recipients in programmatic interventions, protocols, and metrics has historically been limited. Though there is a growing interest in studying stewardship practices in transplant patients, optimal practices have not been clearly established. A component of ASPs, antiviral stewardship (AVS), specifically targeting cytomegalovirus (CMV), has been more recently described. Understanding AVS opportunities and interventions is particularly important for transplant recipients, given the morbidity and mortality associated with viral infections, challenging clinical syndromes, ultrasensitive molecular diagnostic assays, antiviral resistance, and costs of viral disease and medications, as well as antiviral drug toxicities. This review highlights opportunities for AVS for CMV, EBV, HSV, VZV, SARS-CoV-2, respiratory syncytial virus, and BK polyomavirus in transplant patients.

Keywords: antimicrobial stewardship; antiviral stewardship; cytomegalovirus; respiratory syncytial virus

1. Introduction

Antimicrobial stewardship programs (ASPs) promote the appropriate use of antimicrobials with the goal of improving patient outcomes, decreasing unintended adverse effects, and reducing the development of multidrug-resistant organisms (MDROs). Among solid organ transplant (SOT) and hematopoietic-cell transplant (HCT) recipients, antimicrobial stewardship is critical to preserve the efficacy of antimicrobials given the increased risk of both severe and recurrent infections related to underlying immunosuppression. Furthermore, the appropriate use of antimicrobials is essential to avoid drug interactions with immunosuppressive agents and prevent adverse effects on bone marrow, organ, and graft function [1,2].

Historically, ASPs have focused on inpatient settings to provide general guidance on targeted antimicrobial therapy for infections by organ system. This generalized approach is a challenge to apply to transplant recipients as each patient has a unique set of risk factors to consider, including history of colonization with MDROs, viral serostatus, timing since transplant, type of graft, and intensity and duration of immunosuppression. Transplant ASPs build on the goal of optimizing antimicrobial use in both the outpatient and inpatient settings to improve clinical outcomes and decrease unintended adverse effects while considering each patient's unique set of infectious risk factors [1].

While ASPs have primarily addressed the appropriate use of antibacterial and antifungal agents, antiviral stewardship (AVS) in transplant recipients is a growing area of research [3,4]. Though less frequently problematic in immunocompetent patients, common viruses encountered after transplant include cytomegalovirus (CMV), Epstein–Barr virus (EBV), herpes simplex virus (HSV), varicella-zoster virus (VZV), human-herpes virus 6, 7, 8 (HHV-6, HHV-7, HHV-8), and BK polyomavirus (BKPyV). Additionally, widespread community-acquired respiratory viruses such as influenza, respiratory syncytial virus (RSV), adenovirus (AdV), and SARS-CoV-2 may lead to significant morbidity and mortality in immunocompromised patients.

Unfortunately, data evaluating efficacy of antiviral treatment for many of the above viruses that impact transplant recipients are limited to underpowered, uncontrolled studies with heterogenous patient populations, and treatment recommendations are based on expert opinion [5–9]. Additionally, low-level viral replication and detection may not be associated with clinical disease or complications in some populations of transplant recipients (e.g., CMV, HHV-6, BKPyV). In many instances of low-level viral replication, these viruses do not need to be treated. However, the lack of validated viral detection thresholds and heterogeneity of net-immunosuppression across transplant populations (SOT/HCT) further increases the variability of practice.

Many available antivirals commonly used for these viruses may cause significant patient toxicity (ganciclovir, foscarnet, ribavirin, cidofovir). Moreover, prolonged use of valganciclovir/ganciclovir (vGCV/GCV) is associated with (val)ganciclovir-resistant CMV, and emergence of resistance to newer agents like maribavir and letermovir has been described [10–13]. Furthermore, the overuse of antivirals may limit the development of viral-specific cell-mediated immunity leading to late-stage reactivation of viral infections in the setting of increased immunosuppression [14].

Though lack of evidence and limited consensus guidance may lead to management challenges, antiviral drug toxicity, resistance concerns, cost, and the arrival new antivirals on the market make these agents and clinical syndromes prime targets for AVS programmatic interventions. This article will review opportunities to optimize, personalize, or avoid unnecessary antiviral therapy in SOT and HCT recipients focusing on CMV, EBV, HSV/VZV, SARS-CoV-2, RSV, and BKPyV. Though antivirals are recommended for influenza, opportunities for AVS are limited and will not be discussed.

2. Antiviral Stewardship for Cytomegalovirus After Solid Organ Transplantation

Cytomegalovirus remains one the most common infections after SOT and HCT, with direct and indirect effects increasing the risk for other types of infections, impacting graft function, and is associated with significant morbidity and even mortality [15]. Further, CMV continues to challenge patients and clinicians in the current era of prophylaxis, rapid molecular tests, and CMV-specific cell-mediated immunity (CMI) assays.

The most comprehensive CMV-specific stewardship program recently described by Jorgensen et al. was initiated amidst institutional concerns regarding CMV resistance, related hospital admissions, prolonged admissions, associated costs, graft outcomes, and antiviral toxicities [3]. This program was modeled on traditional ASPs and was supported by a multidisciplinary group of clinicians from pharmacy, transplant ID, and abdominal transplant physicians and surgeons, and relied heavily on prospective audit and feedback [4]. Consensus guidelines were created for prophylaxis and treatment agents, dosing and duration, and screening and diagnosis incorporating CMV cell-mediated immunity (CMI) testing to dictate the need for further surveillance. This CMV stewardship bundled intervention significantly decreased the valganciclovir days of therapy (DOT) as well as the rate of ganciclovir-resistant CMV over time [3].

2.1. CMV Prevention

Prophylaxis and pre-emptive therapy (PET) are acceptable CMV prevention strategies, with PET mostly utilized after HCT [15]. However, centers may consider PET as a stewardship-concordant strategy in SOT, with recent data in high-risk liver transplant recipients supporting better CMV-specific outcomes, including CMV immunologic endpoints and 5-year survival [16–18].

Though historically vGCV has been the most widely used antiviral for primary CMV prophylaxis after SOT, a recent randomized, multicenter phase 3 trial evaluated kidney transplant recipients with high-risk CMV serostatus (D+/R−), comparing primary prophylaxis with letermovir (with acyclovir) to vGCV, and found that letermovir was noninferior to vGCV, with lower rates of leukopenia and neutropenia [19]. Real-world experience with letermovir primary prophylaxis in other SOT populations (e.g., heart, lung transplant) has

been described in small retrospective studies and may be helpful in patients unable to obtain or tolerate vGCV [20,21]. The benefits of letermovir prophylaxis need to be weighed against other factors including cost, access, adverse effects, and drug interactions [22–24]. Reported adverse effects related to letermovir include nausea, vomiting, diarrhea, edema, and atrial fibrillation or flutter [22,25]. There are multiple drug interactions to consider when using letermovir as it decreases voriconazole and increases tacrolimus, sirolimus, and cyclosporine levels [23,24]. Larger studies, including cost-effectiveness analyses, are needed to understand and refine ideal letermovir prophylactic strategies in SOT. Until more is understood, targeting inappropriate letermovir use may be an AVS opportunity for many transplant centers. Importantly, letermovir does not have activity against HSV or VZV, and prophylactic agents active against these viruses, e.g., (v)ACV, should be added if indicated.

Preventing CMV recurrence after therapy may be challenging in SOT recipients, and some centers routinely give secondary antiviral prophylaxis to all transplant recipients. Though no prospective, randomized trials have been carried out to evaluate the role of secondary prophylaxis to prevent CMV recurrence, multiple retrospective studies have demonstrated no benefit [26–28]. Further, national and international consensus guidelines recommend against the routine use of secondary prophylaxis for all patients [15,27]. These recommendations, combined with the potential antiviral toxicity, resistance, and cost, make secondary prophylaxis an ideal AVS target to address.

2.2. CMV Treatment

The currently available antiviral drugs for the treatment of CMV infection and disease are limited by numerous adverse effects. First-line therapy, vGCV, and GCV have unintended myelosuppressive effects, which is associated with poor outcomes in SOT [29]. Furthermore, as their administration requires dose adjustments based on renal function, exposure to subtherapeutic levels leads to the development of drug-resistant CMV [15]. Foscarnet is a recommended second-line agent for CMV treatment if patients are intolerant to vGCV/GCV, or it may be used for ganciclovir-resistant viruses. Lastly, cidofovir is associated with nephrotoxicity, electrolyte wasting, and neurotoxicity and should be avoided after SOT if possible [30].

Maribavir, a UL97 protein kinase inhibitor with a good safety profile, is a newer option for CMV in SOT. Maribavir is approved for the treatment of refractory/resistant (R/R) CMV infection or disease in SOT and HCT recipients. In the SOLSTICE trial, a phase 3, open-label study of 352 HCT and SOT recipients with R/R CMV randomized to maribavir or investigator-assigned therapy (IAT; valganciclovir/ganciclovir, foscarnet, cidofovir), maribavir was significantly more effective at CMV viremia clearance and symptom control along with fewer treatment discontinuations due to adverse effects compared to IAT [31]. Small, single-center reports of maribavir use in SOT have been published but defining indications and understanding utilization are programmatic AVS responsibilities [32]. Importantly, maribavir does not have activity against HSV or VZV, and prophylactic agents active against these viruses, e.g., (v)ACV, should be added if indicated.

Intravenous (IV) to oral (PO) conversion of antimicrobials is a main function of ASPs to avoid complications of intravenous therapy and decrease length of hospital stay [33]. With strong evidence from the VICTOR trial establishing noninferiority of valganciclovir compared to IV ganciclovir for the treatment of CMV, IV to PO conversion of valganciclovir is an additional opportunity for AVS in SOT [34]. Though case reports of antiviral therapeutic drug monitoring (TDM) have been described in SOT recipients, the lack of test availability and clinical guidance for therapeutic targets make this strategy an exciting area for potential future investigation [35].

2.3. Laboratory Opportunities

Working with the local laboratory and transplant team to establish and protocolize diagnostic and screening guidance and virologic thresholds for CMV treatment may be a

diagnostic stewardship opportunity for AVS programs [15]. An outpatient, pharmacy-led intervention consisting of real-time CMV PCR surveillance and result notification coupled with drug therapy optimization recommendations resulted in more patients with CMV clearance and lower peak CMV viral loads [36]. Additionally, appropriate CMV resistance testing (UL97 and UL54) should only be routinely sent if DNAemia persists or increases, or if CMV-related clinical symptoms do not resolve after at least 2 weeks of appropriately dosed (v)GCV therapy [27]. Though the routine clinical role of CMV CMI testing may not be solidly established, opportunities to assess risk and personalize treatment for primary and secondary prophylaxis show promise as a future AVS tool [37,38]. Unfortunately, widespread protocolization and recommendations for testing are further challenged by the lack of commercial availability of certain CMV CMI assays (i.e., QuantiFERON-CMV) in the US. Antiviral stewardship opportunities and challenges in CMV in SOT are summarized in Table 1.

Table 1. Antiviral stewardship challenges and opportunities in cytomegalovirus in solid organ transplantation.

Focus Area	Challenges	Opportunities
Pre-emptive therapy (PET)	vGCV prophylaxis most common strategy. PET is resource-intensive.	PET personalized therapy, improved CMV, immunologic, mortality endpoints vs. prophylaxis in high-risk liver transplant.
Letermovir prophylaxis	Limited evidence outside of kidney transplant. Cost, availability, drug interactions.	Prophylaxis targeted to groups intolerant of vGCV, cytopenias.
Secondary prophylaxis	Recurrent DNAemia/disease may occur after therapy cessation.	Secondary prophylaxis should be avoided generally. Consider for high-risk patients with severe disease. CMI monitoring may be helpful.
Drug administration	Moderate–severe disease may require hospitalization.	IV to oral transition should be performed in most scenarios.
Therapy	vGCV/GCV with myelosuppressive effects, renal dose adjustment. Foscarnet with nephrotoxicity, electrolyte wasting.	Maribavir less myelosuppressive and nephrotoxic. Consider for R/R disease, GCV intolerance, and cytopenias.

PET: pre-emptive therapy; vGCV: valganciclovir; GCV: ganciclovir; CMI: cell-mediated immunity; R/R: refractory/resistant.

3. Antiviral Stewardship for Cytomegalovirus After Hematopoietic Stem Cell Transplant

3.1. CMV Prevention

Though CMV is a frequently encountered viral infection after allo-HCT associated with significant morbidity and mortality, to our knowledge there has been no comprehensively described AVS or CMV stewardship program described in the HCT population [39]. The American Society for Transplantation and Cellular Therapy (ASTCT) guidelines recommend two complementary strategies for CMV prevention in CMV seropositive patients after allo-HCT in the early post-engraftment period: CMV monitoring for pre-emptive therapy (PET) and chemoprophylaxis with letermovir [40]. In a landmark phase 3, double-blinded, randomized control trial of 495 CMV seropositive allo-HCT recipients by Marty et al., patients on letermovir prophylaxis through to week 14 had a significantly lower risk of clinically significant CMV infection compared to the placebo group at the week-24 follow-up [22]. The impact of letermovir on the prevention of CMV infection and mortality was even more pronounced among patients at high risk for CMV reactivation [22].

An additional factor to consider with the use of letermovir is the potential delay in the development of CMV-specific T-cell-mediated immunity (CMV-CMI) leading to late-stage

CMV reactivation after discontinuation of prophylaxis. In the landmark trial by Marty et al., clinically significant CMV infection was reported in 10% of all patients and 20% of patients at high risk for CMV infection at the 24-week follow-up, 10 weeks after letermovir prophylaxis was discontinued [22]. Similar findings were demonstrated in a phase 3, multicenter, double-blinded, randomized control trial of extended-duration letermovir prophylaxis in 220 CMV seropositive allo-HCT recipients at high risk of late CMV infection by Russo et al. [25]. Extending letermovir prophylaxis from day 100 to day 200 was safe and effective in reducing the risk of clinically significant CMV infection; however, there was no significant difference in the incidence of clinically significant CMV reactivation by the 48-week follow-up, 20 weeks after letermovir prophylaxis was discontinued [25]. The late-stage CMV reactivation in both trials was likely related to delays in the development of CMV-CMI. Zamora et al. demonstrated that patients on letermovir prophylaxis had decreased CMV-specific T-cell responses to multiple CMV antigens (immediate early-1 and phosphoprotein 65) compared to the PET group, possibly related to the suppression of CMV replication and decreased exposure to CMV antigens [14].

A stewardship initiative to optimize letermovir use after allo-HCT involves targeting chemoprophylaxis for patients at high risk for CMV infection. High-risk patients are defined as CMV seropositive allo-HCT recipients having one or more of the following criteria: mismatched donor, haploidentical donor, use of umbilical cord blood as stem-cell source, and the use of ex vivo T-cell depleted grafts [22,25,41]. A recent cost analysis comparing letermovir prophylaxis and PET through day +180 after allo-HCT found that letermovir was associated with significant reductions in CMV-related admissions and decreased the median total cost of care in high-risk CMV patients [42]. Among patients at low risk for CMV reactivation not meeting any of the above criteria, CMV monitoring with PET may be a more beneficial preventative strategy to allow exposure to CMV antigens for the development of CMV-CMI.

Building on AVS strategies for CMV prevention in SOT recipients by Jorgenson et al., a proposed clinical algorithm involves routine monitoring of CMV-CMI in the early post-engraftment period to distinguish between allo-HCT recipients with ongoing risk of developing CMV infection and those who are protected [3]. The REACT study demonstrated that allo-HCT patients with high CMV-CMI, as determined by a peptide-based enzyme linked immunospot (ELISPOT) CMV assay, were less likely to develop clinically significant CMV infection [43]. Once CMV-CMI develops during routine monitoring after allo-HCT, CMV prevention strategies, including letermovir prophylaxis and CMV monitoring with PET, can likely be discontinued.

3.2. CMV Treatment

Similarly to SOT, the available therapies for CMV infection and disease in HCT are also limited by numerous adverse effects. The myelosuppressive effects of vGCV/GCV may require support with granulocyte colony-stimulating factor and are generally avoided during the pre-engraftment stage, and underdosing in HCT is a risk for development of drug-resistant CMV [44]. Foscarnet is recommended for CMV treatment during the pre-engraftment stage, but toxicities are also seen in HCT patients [45]. Cidofovir, reserved for third-line therapy, should be avoided when possible [30].

Extrapolating from the results of the VICTOR trial, many subsequent studies have demonstrated that valganciclovir has equivalent clinical efficacy to ganciclovir for CMV pre-emptive therapy in HCT recipients [34,44,46]. Even in the setting of mild intestinal graft-versus-host-disease (GVHD), valganciclovir achieves equivalent serum levels to ganciclovir [47]. Therefore, intravenous GCV to oral vGCV conversion may also be a target for AVS activities in HCT recipients.

Based on the results of the SOLSTICE trial, maribavir is a promising agent for CMV PET after allo-HCT [31]. Unfortunately, maribavir may not be as effective as vGCV for the treatment of first asymptomatic CMV infection after allo-SCT. In the AURORA trial, a multicenter, double-blind, phase 3 study of 547 HCT recipients with first asymptomatic CMV

infection randomized to maribavir or valganciclovir, maribavir did not meet the primary endpoint of noninferiority for CMV viremia clearance (maribavir 69.9%, valganciclovir 77.4%) at week 8. However, patients treated with maribavir maintained CMV clearance without tissue-invasive disease at week 16 post-treatment follow-up. The maribavir group was less likely to develop neutropenia and require treatment discontinuation from adverse effects [48]. Using hospital LOS data from the SOLSTICE trial and modeling CMV-related costs from the published literature, a recent US-based analysis led by maribavir's sponsor estimated that maribavir was likely associated with cost-savings when compared to IAT for R/R CMV [49]. Additional studies are needed to determine the role of maribavir in the management of CMV infection in allo-HCT recipients.

CMV adoptive T-cell therapy is another promising treatment for the management of CMV infections, particularly in the setting of refractory and resistant CMV disease after HCT. Several nonrandomized clinical studies have demonstrated the safety and efficacy of donor-derived or third-party CMV-specific T-cells (CTLs) [50]. Unfortunately, CTLs require a lengthy and expensive process of identifying HLA-matched CMV seropositive donors with subsequent isolation of CMV-specific T-cells for transfusion. Recent advances include the development of "off-the-shelf" third-party CTLs banks [51,52]. While CMV cellular therapies are currently limited by cost and availability, their widespread use could help avoid adverse drug effects and restore CMV immunity to prevent late-stage reactivation. However, the role of these therapies is currently uncertain, and experiences should be reported as further studies are needed. Antiviral stewardship challenges and opportunities in CMV after HCT are summarized in Table 2.

Table 2. Antiviral stewardship challenges and opportunities in cytomegalovirus in allogeneic hematopoietic cell transplantation.

Focus Area	Challenges	Opportunities
Letermovir prophylaxis	Delays CMV-CMI with late-stage reactivation. Cost, availability, drug interactions.	Prophylaxis targeted to groups with high risk of CMV infection. Duration of prophylaxis guided by CMV-CMI.
Drug administration	Oral vGCV absorption in setting of gastrointestinal disease and GVHD.	Transition from intravenous to oral therapy with GVHD stage $\leq$ 2.
Therapy	vGCV and GCV with myelosuppressive effects, requires renal dose adjustment. Foscarnet with nephrotoxicity, electrolyte wasting.	Maribavir less myelosuppressive and nephrotoxic. CTLs may restore CMV-CMI and prevent late-stage reactivation; role in AVS needs further definition.

CMV-CMI: cytomegalovirus T-cell-mediated immunity; GVHD: graft-versus-host disease; CTL: CMV-specific T-cells.

4. Antiviral Stewardship for Respiratory Syncytial Virus

RSV is a frequently encountered community-acquired respiratory virus in transplant recipients associated with significant morbidity and mortality in immunocompromised hosts [53,54]. Among allo-HCT and lung transplant recipients, RSV upper respiratory infections (URIs) are more likely to progress to lower respiratory tract disease (LRD) with resulting respiratory failure and death [55]. Lung transplant recipients can also suffer from chronic sequelae after severe RSV infections as immune dysregulation can lead to chronic lung allograft dysfunction [56]. Ribavirin is a nucleoside analogue with in vitro activity against RSV. As there are no large prospective randomized double-blinded clinical trials on the safety and efficacy of ribavirin, there is no consensus on the optimal treatment of RSV in HCT and SOT recipients [53,54]. Based on data from observational studies, current clinical guidelines recommend considering the use of ribavirin in severely immunocompromised patients where treatment may reduce progression to LRD and death [53,54].

An important AVS initiative is reserving the use of ribavirin for patients who would receive the most clinical benefit from treatment as the drug is associated with hematologic

toxicities including anemia, hemolytic anemia, neutropenia, and lymphopenia [57]. Among allo-HCT recipients, patients who are severely immunocompromised when presenting in the earliest stages of RSV URI may benefit the most from ribavirin therapy. Shah et al. developed the Immunodeficiency Scoring Index (ISI)-RSV that stratified allo-HCT recipients into three risk groups (low, moderate, and high) based on six immunodeficiency markers immediately available on routine bloodwork: age $\geq$ 40 years, absolute neutrophil count (ANC) < 500/μL, absolute lymphocyte count (ALC) < 200/μL, myeloablative conditioning regimen, acute or chronic GVHD, corticosteroids, and recent or pre-engraftment allo-HCT. The study found that in a cohort of 237 allo-HCT recipients, those with high risk based on the ISI-RSV benefited the most from ribavirin when administered during the URI stage, and they had the highest risk of progression to LRD and death when ribavirin was not initiated [58].

An AVS initiative to optimize ribavirin use identifies allo-HCT recipients early in the RSV disease course and reserves ribavirin for patients determined to be at high risk for severe disease. For a timely diagnosis of RSV, all allo-HCT recipients presenting in the outpatient or inpatient setting with URI symptoms would immediately be tested using a multiple polymerase chain reaction (PCR) respiratory viral panel. The decision to treat would be based on the patient's risk for progression to LRD using the ISI-RSV. Additional risk factors for progression from URI to LRD to consider when deciding to initiate ribavirin include history of smoking, total body irradiation > 1200 cGy, use of a mismatched or unrelated donor, multiple transplantations procedures, prior exposure to antibiotics, and serum glucose > 200 mg/dL [53]. Transplant centers can consider incorporating these risk factors into treatment algorithms to assist with clinical decision making.

Another AVS opportunity involves replacing aerosolized ribavirin with the oral formulation for the treatment of RSV in severely immunocompromised hosts. The initial studies demonstrating a potential clinical benefit of ribavirin used the aerosolized formulation for the treatment of RSV [59–61]. However, there are many drawbacks to aerosolized ribavirin, including the risk of bronchospasm, pulmonary edema, dyspnea, and impaired ventilation and oxygenation in the patient. An additional limitation to ribavirin includes its potential teratogenicity to healthcare providers exposed to the drug. Administration of aerosolized ribavirin requires a scavenging tent, a process that is challenging for the respiratory therapist and uncomfortable for patients who may already be experiencing respiratory distress [62]. The decision to initiate treatment with aerosolized ribavirin is challenging as clinicians must weigh the potential risks with unknown clinical benefit given the minimal evidence supporting its use.

Subsequent research has demonstrated that oral and aerosolized ribavirin formulations have similar efficacy in the treatment of RSV for both allo-HCT and lung transplant recipients [63,64]. A retrospective cohort study of 124 allo-HCT recipients with RSV infection found that the rate of progression to LRD and 30-day mortality were similar among those treated with oral and nebulized ribavirin [63]. In a retrospective study of 52 lung transplant recipients with symptomatic RSV infection, oral ribavirin was found to be an effective and well-tolerated alternative to inhaled ribavirin with associated cost savings and reduced length of hospital stay [64]. Restricting adult hospital formularies to oral ribavirin may decrease healthcare costs and prevent adverse effects in patients and healthcare workers.

The US Food and Drug Administration has approved multiple safe and effective vaccines for the prevention of RSV LRD in adults $\geq$ 60 years: adjuvant monovalent vaccine Arexvy, nonadjuvanted bivalent vaccine Abrysvo, and the mRNA vaccine mResvia [65]. Widespread immunization of immunocompromised patients at high risk for LRD is the most impactful stewardship intervention to decrease the incidence of symptomatic RSV, prevent hospitalizations, and obviate the need for ribavirin. As the vaccines clinical trials did not include immunocompromised hosts, there are no clinical guidelines on their use among SOT and HCT recipients [66–68]. The immunogenicity of RSV vaccine among immunocompromised hosts is an active area of research that will guide future recommendations on the optimal age of vaccine initiation and the frequency of boosters.

Based on the available information, an AVS strategy to increase RSV vaccination rates involves the routine recommendation of RSV vaccines during pretransplant counseling visits to ensure that vaccines are administered prior to the onset of immunosuppression. For pre- and post-transplant patients $\leq$ 60 years, shared clinical decision making between the clinician and the patient is encouraged to discuss the potential benefits while weighing the possible out-of-pocket cost for the vaccine. Considerations for AVS for RSV after transplantation are summarized in Table 3.

Table 3. Antiviral stewardship challenges and opportunities in respiratory syncytial virus.

Target Area	Challenges	Opportunities
Rapid diagnostics	Ribavirin therapy less effective at LRD stage.	Clinical treatment guideline for alloHCT/lung transplant presenting with URI symptoms with prompt testing using multiplex PCR respiratory viral panel.
Targeted treatment	Minimal benefit of ribavirin in low- and moderate-risk ISI-RSV patients.	Treatment algorithm reserving ribavirin treatment for alloHCT/lung transplant with high-risk patients ISI-RSV.
Drug administration	Toxicity of nebulized ribavirin to patients and healthcare providers.	Replace aerosolized with oral ribavirin in adult hospital formularies.
Vaccine	Lack of efficacy data on immunocompromised hosts.	Shared clinical decision making during pre-SOT and HCT evaluations.

LRD: lower respiratory tract disease; URI: upper respiratory tract; PCR: polymerase chain reaction; ISI: Immunodeficiency Scoring Index; SOT: solid organ transplant; HCT: hematopoietic cell transplant.

4.1. Antiviral Stewardship for SARS-CoV-2

Immunosuppression has been associated with an increased risk of COVID-19-related hospitalizations in SOT recipients [69]. While a majority of HCT and SOT patients appear to have lower disease severity from infections with current variants, certain groups, such as lung transplant recipients, may still experience severe COVID-19 [70,71]. Unfortunately, resistance development on therapy has been described in immunocompromised patients [72].

Transplant recipients were largely excluded from early-pandemic, antiviral trials, and large, robust studies in transplant populations are lacking. There have been prospective and retrospective studies evaluating antiviral interventions (remdesivir, nirmatrelvir/ritonavir, molnupiravir) in transplant recipients with COVID-19 [73–75]. The Infectious Diseases Society of America (IDSA) treatment guidelines do not give specific antiviral treatment recommendations for transplant recipients, though currently available guidance from transplant experts is similar [76–78]. For patients with mild to moderate COVID-19, guideline-recommended options are remdesivir (3 days), nirmatrelvir–ritonavir, or molnupiravir, or neutralizing monoclonal antibodies and 5 days of remdesivir if patients have severe COVID-19 and are on supplemental oxygen. Though nirmatrelvir-ritonavir has been safely used in SOT and HCT recipients, ritonavir's strong inhibition of cytochrome P450 3A4 leads to a substantial interaction with calcineurin inhibitors (CNIs), which may lead to severe toxicities [75].

The changing variant landscape and immunity conferred from prior infection and SARS-CoV-2 vaccination further challenges understanding of the most ideal patient, time, and setting of antiviral interventions. AVS initiatives for SARS-CoV-2 may focus on institutional protocols supporting clinicians to understand an individual SOT/HCT recipient's risk of severe disease and subsequent need for antivirals in low-risk, vaccinated patients with mild symptoms. Additionally, ensuring appropriate durations of remdesivir based on disease severity (3 days mild–moderate; 5 days for severe) may be another opportunity for AVS monitoring. Working with transplant pharmacy teams to support management and patient education regarding nirmatrelvir–ritonavir CNI interactions may also be beneficial. To avoid unnecessary antivirals, AVS for SARS-CoV-2 should include diagnostic stewardship, avoiding screening of asymptomatic patients in most circumstances in the pre- and

post-transplant setting. The use of immunomodulatory agents for patients with severe COVID-19 may also be an opportunity for AVS support, though this is beyond the scope of this review. Considerations for AVS for SARS-CoV-2 after transplantation are summarized in Table 4.

Table 4. Antiviral stewardship challenges and opportunities in SARS-CoV-2.

Target Area	Challenges	Opportunities
Rapid diagnostics	SARS-CoV-2 NP swab screening of asymptomatic patients may lead to over treatment.	Diagnostic stewardship limiting SARS-CoV-2 screening in asymptomatic patients in the pre- and post-transplant setting.
Targeted treatment	Unclear benefit of therapy in asymptomatic or mildly symptomatic patients.	Treatment algorithm reserving treatment for patients with significant symptoms who are high risk.
Drug administration	Remdesivir must be given intravenously.	Appropriate durations of remdesivir based on disease severity (3 days mild–moderate; 5 days severe).
Drug–drug interaction	Nirmatrelvir–ritonavir strong inhibitor of cytochrome P450 3A4, causing CNI interaction.	Educate patients and providers to avoid CNIs in SOT recipients unless close monitoring available.

NP: nasopharyngeal; SOT: solid organ transplant; CNI: calcineurin inhibitor.

4.2. Antiviral Stewardship for HSV/VZV

After SOT, most recipients receive vGCV prophylaxis, which generally provides protection against HSV and VZV reactivations. In some situations, (v)ACV may be given instead of vGCV (toxicity, cost, CMV D-/R-) for a recommended duration of at least 1 month or after episodes of rejection [79]. After HCT, HSV risk is highest in the early post-transplant period. ACV prophylaxis decreases HSV and VZV reactivation [80]. The National Comprehensive Cancer Network (NCCN) recommends HSV prophylaxis for a minimum of 2 months after alemtuzumab and until CD4 $\geq$ 200 cells/mcL and VZV prophylaxis for at least 1 year after allogeneic HCT [81].

Optimizing institutional protocols for prophylaxis and treatment and attempting to monitor prescription indications for (v)ACV is an additional opportunity for AVS. Further, in coordination with transplant pharmacy clinicians, AVS should work to ensure that patients who are prescribed letermovir for CMV prophylaxis (after SOT or HCT) and/or maribavir for refractory/resistant CMV therapy are also on an HSV/VZV-active agent such as (v)ACV if they meet the criteria to receive HSV/VZV prophylaxis.

Empiric ACV for patients with presumed HSV or VZV encephalitis is commonly encountered in clinical practice. Studies including transplant and immunocompromised patients have reported decreases in ACV utilization after implementation of meningitis encephalitis panels, which may support AVS [82,83]. However, it is important to keep in mind that false-negative HSV PCR results have been reported from meningitis encephalitis panels, and HSV 1/2-specific PCR testing should be considered if concern for infection remains [84].

4.3. Antiviral Stewardship for EBV

In patients who have undergone SOT or HCT, EBV reactivation is common and can be associated with a variety of clinical syndromes. However, EBV-associated post-transplant lymphoproliferative disorder is the most concerning and challenging. Though monitoring EBV DNAemia is common after SOT and HCT, relevant thresholds dictating interventions and predicting PTLD across different organs transplanted, as well as variability across labs, further challenges EBV management in transplantation. EBV DNAemia monitoring for allo-HCT recipients is recommended to begin within the first month post-transplant and continue weekly for at least 4 months and longer if patients are suspected to have poor T-cell reconstitution [85]. In EBV-negative SOT recipients of EBV seropositive donors, EBV DNA monitoring is recommended weekly to biweekly in the first year after transplant [86].

In patients who develop EBV DNAemia (center-defined threshold), weekly rituximab is the first-line agent for preemptive therapy and is given weekly for 1–4 doses or until EBV DNAemia negativity and should be combined with immunosuppression reduction when possible [85]. In SOT recipients with EBV DNAemia, a reduction in immunosuppression is the first-line recommended management strategy, as data using rituximab therapy are limited [86]. EBV DNA monitoring after HCT or mismatched SOT may be an opportunity for diagnostic stewardship. Optimization of rituximab prescription may also be supported by AVS programs targeting center-specific guideline adherence in order to decrease practice variability.

While ACV and GCV have in vitro activity against lytic forms of EBV, and the lytic cycle is associated with the progression of EBV tumors, latent EBV-driven lymphoproliferation predominates in PTLD lesions [87]. However, ACV and GCV have no impact on latent EBV infection. Though some single- and multicenter retrospective studies suggest that antiviral prophylaxis reduces PTLD incidence, larger, more methodologically robust studies demonstrate otherwise [86]. Therefore, the use of antivirals, (v)ACV and (v)GCV, for prophylaxis or preemptive therapy to prevent EBV-positive PTLD is not recommended after SOT or HCT [85,86]. Similarly, there is insufficient evidence to support the routine use of antiviral therapy for the treatment of EBV-positive PTLD after SOT or HCT [85,86]. Considering that the totality of data does not support antivirals for EBV, and national and international guidelines recommend that antivirals should not be used, antiviral utilization for the prevention or treatment of EBV may be an opportunity for AVS through the creation of institutional guidance and monitoring drug utilization for this indication. If available, cellular therapy, such as EBV-specific CTLs, is recommended to prevent and treat PTLD after HCT but not after SOT, given limited data [85,86]. The role of CTLs in AVS has not yet been determined and needs further study. Emerging diagnostics, such as those testing for the Z EBV replication activator (ZEBRA) protein, identifying lytic virus, have been studied in transplant recipients, and newer strategies to inhibit EBV lytic replication are being explored [88,89]. The future role for these diagnostic and therapeutic interventions within AVS programs remains to be determined. Considerations for AVS for EBV after transplantation are summarized in Table 5.

Table 5. Antiviral stewardship challenges and opportunities in EBV.

Target Area	Challenges	Opportunities
Diagnostics	EBV DNAemia recommended in high-risk patients, no defined threshold for preemptive interventions.	Institutional protocol for EBV DNA monitoring and threshold validation.
Management	For EBV DNAemia: RIS primary strategy in SOT; rituximab primary in HCT.	Monitor appropriateness of rituximab therapy in HCT.
Antivirals	Antivirals (ACV, GCV) with in vitro activity; limited evidence for prevention or treatment of EBV/PTLD.	Monitor antivirals used to treat EBV/PTLD.
Cellular therapy	EBV CTLs recommended in HCT, not SOT.	If available, institutional protocol for CTLs; role in AVS needs further definition.

EBV: Epstein–Barr virus; RIS: reduction in immunosuppression; HCT: hematopoietic cell transplant; SOT: Solid organ transplantation; ACV: acyclovir; GCV: ganciclovir; CTLs: EBV-specific T-cells; AVS: antiviral stewardship.

4.4. Antiviral Stewardship for BK Polyomavirus in Transplantation

Lowering immunosuppression is the primary recommended management strategy for BKPyV DNAemia or related disease. Though cidofovir has in vitro activity against BKPyV, there are no prospective, well-controlled trials demonstrating clinical efficacy, and, ideally, it should be avoided in kidney transplant recipients where BKpyV is most commonly managed [90]. Recently updated international guidelines recommend against the use of cidofovir for BKPyV DNAemia or nephropathy in kidney transplant recipients [91]. Addi-

tionally, though past clinical reports of fluoroquinolones' impacts on BK-related disease have been mixed, a more recent systematic review and two prospective RCTs found that quinolones did not prevent BKPyV DNAemia or disease and, in one study, were associated with increased rates of fluroquinolone-resistant infections [92–94]. Consequently, recent guidelines recommend against the use of fluroquinolones to prevent or treat BKPyV DNAemia or nephropathy after kidney transplantation [91]. Similarly, systemic cidofovir has been evaluated for the treatment of BKPyV-associated hemorrhagic cystitis in patients after HCT in small, observational studies with mixed results and should not be routinely used [5,95].

5. Potential Antiviral Stewardship Program Metrics

Evaluating and reporting metrics are recommended for ASPs in the general population, though transplant-specific metrics, particularly ideal AVS measures, are not as well understood [33]. Results from a 2015 survey by Seo et al. found that 23% of transplant programs that included SOT/HCT in ASPs did not track outcome metrics in these patient groups [96]. Metrics used in the comprehensive CMV stewardship program described by Jorgenson et al. included antiviral utilization, rates of GCV-resistant CMV, and hospital admission and duration for IV foscarnet [3]. AVS process metrics may focus on institutional guideline adherence, diagnostic optimization, and appropriateness of antivirals (e.g., inhaled or PO ribavirin, letermovir, and maribavir). Outcome and balancing metrics such as CMV DNAemia and disease recurrence, related hospitalizations, adverse drug events, and resistance are more resource-intensive and may be more challenging to monitor. Examples of antiviral stewardship program metrics are summarized in Table 6.

Table 6. Examples of potential antiviral stewardship program metrics.

Process	Outcome	Balancing
Usage (DOT, LOT)	Infection rate: CMV DNAemia and disease.	Infection rate.
Guideline adherence	Virus-related hospitalization.	Virus-related readmission.
Intervention acceptance rate	Adverse drug event.	Readmission.
Diagnostic appropriateness (CMV PCR, CMI testing)	Resistance rate.	Recurrent DNAemia/disease after treatment.
	Time to CMV eradication.	Infectious mortality (CMV/RSV).

DOT: days of therapy; LOT: length of therapy; CMI: cell-mediated immunity.

6. Conclusions

There are significant opportunities for AVS in transplant recipients to optimize patient outcomes. Though AVS can be easily integrated with institutional ASPs, further research on program structure, optimal interventions, and valuable metrics is needed.

Author Contributions: Conceptualization: S.B., S.T. and J.H.; original draft preparation: S.B., S.T. and J.H.; review and editing, J.H.; supervision, J.H. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Conflicts of Interest: S.B.—none; S.T.—none; J.H. grant/research: Pfizer, Janssen, Rebiotix, Scynexis, AstraZeneca, ARLG, NIH/NIAID; Advisory Board: Pfizer, AstraZeneca, Innoviva; Honoraria: The Antibiotic Resistance Leadership Group (ARLG).

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Review

Cytomegalovirus Infection After Solid Organ Transplantation: How I Use Cell-Mediated Immune Assays for Management

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Abstract: Introduction: The pathogenesis and outcome of cytomegalovirus (CMV) infection after solid organ transplantation (SOT) reflects the interplay between viral replication and CMV-specific immunity. Despite advances in its diagnosis and treatment, CMV continues to cause significant morbidity after SOT. Since CMV is an opportunistic pathogen that occurs as a result of impaired pathogen-specific immunity, laboratory assays that measure CMV-specific immune responses may be useful in assisting clinicians in its management. Methods and Results: The author summarizes the evolving and emerging data on the clinical utility of assays that quantify cell-mediated immune responses to CMV in SOT recipients. The majority of publications are observational studies that demonstrate that a lack or deficiency in CMV-specific cell-mediated immunity is correlated with a heightened risk of primary, reactivation, or recurrent CMV after transplantation. A few prospective interventional studies have utilized CMV-specific cell-mediated immune assays in guiding the duration of antiviral prophylaxis among CMV-seropositive SOT recipients. Likewise, CMV-specific cell-mediated immunity assays have been suggested to inform the need for secondary antiviral prophylaxis and immunologic optimization to prevent CMV relapse after treatment. Conclusions: CMV-specific cell-mediated immune assays are emerging to assist transplant clinicians in predicting a patient's risk of CMV after transplantation, and these assays have been utilized to individualize the approach to CMV prevention and treatment. The author suggests the conduct of more interventional studies to further solidify the role of CMV-specific cell-mediated immune assays in routine clinical practice.

Keywords: cytomegalovirus; cell-mediated immunity; interferon gamma; transplantation; solid organ transplantation; immunosuppression; antiviral therapy; relapse; prophylaxis; outcomes

1. Introduction

Cytomegalovirus (CMV) is a ubiquitous herpesvirus that infects the majority of adults [1]. When an immunocompetent person acquires CMV infection, it is generally a self-limiting, non-specific illness that spontaneously resolves without antiviral therapy. CMV-specific cellular and humoral immunity develops during primary CMV infection, characterized by the generation of CMV-specific CD4+ and CD8+ T lymphocytes and CMV-IgM and IgG antibodies, respectively [2]. However, CMV persists lifelong in humans, with periodic reactivations, but the virus is kept in a latent subclinical state by a functioning cell-mediated immune system [3].

In persons with compromised immune systems, primary or reactivation CMV infection can lead to high morbidity and, if untreated, potential mortality. In the field of solid organ transplantation (SOT), where patients are given lifelong pharmacologic immunosuppression to prevent allograft rejection and ensure the survival of the transplanted allograft, CMV has the potential to cause severe disease [4]. The major target of most immunosuppressive drugs used in SOT recipients is the T lymphocyte, although other immune cells, such as natural killer (NK) cells, may also be affected. Some immunosuppressive drugs

deplete T lymphocyte populations (lymphocyte-depleting drugs; e.g., thymoglobulin, alemtuzumab), while others impair lymphocyte function (e.g., basiliximab) [5]. SOT recipients are maintained lifelong on a combination of pharmacologic immunosuppressive drugs that impair the function of T lymphocytes and other immune cells, such as calcineurin inhibitors (tacrolimus, cyclosporine), antimetabolites (mycophenolate, azathioprine), and corticosteroids [4]. While these drugs are intended to ensure allograft survival, one major adverse outcome is a heightened risk of opportunistic infections, including CMV.

CMV infection is a common complication after SOT, and it can either be primary infection or reactivation of latent virus [4]. Primary CMV infection can be potentially severe and life-threatening in SOT recipients. Most often, primary CMV infection occurs when CMV-seronegative transplant patient receives an organ from a CMV-seropositive donor (CMV D+/R− mismatch) [4]. Among CMV-seropositive SOT recipients with pre-existing immunity, latent CMV may reactivate to cause clinical disease when there is depletion or marked impairment in the function of T lymphocyte populations [4]. CMV disease may be manifested as fever associated with malaise, leukopenia, thrombocytopenia, and mild hepatic transaminitis (CMV syndrome) [4]. In many patients, CMV may be disseminated to various organs to cause dysfunction, including the transplanted allograft. The gastrointestinal tract is the most commonly affected organ system [6], while CMV pneumonitis can be particularly severe, morbid, and potentially fatal [7]. CMV may affect any organ system, including the central nervous system (CNS) and the retina (i.e., retinitis) [8]. CMV has been correlated with poor allograft and patient survival after SOT [9].

Because of the negative effects of CMV in SOT, its prevention is part of standard of care. This can be accomplished either through antiviral prophylaxis or preemptive therapy [4]. Antiviral prophylaxis entails the administration of an antiviral drug, most commonly valganciclovir or letermovir, for a defined period (usually as short as 3 months to as long as 12 months, or longer) after SOT [4]. In contrast, preemptive therapy is a strategy that entails antiviral drug administration only upon the detection of asymptomatic CMV replication. Using this strategy, patients are monitored regularly, often once weekly, via CMV nucleic acid amplification testing (e.g., CMV DNA polymerase chain reaction [PCR]) of blood samples during the first 3 months after SOT. If CMV DNA is detected above a pre-specified viral load threshold, antiviral drugs such as valganciclovir are provided to treat the patients until the virus is no longer detectable in the blood [4].

The risk, clinical course, and outcome of CMV in SOT is dependent on the interplay between virus replication and host immune fitness. Virus replication is measured via quantitative CMV nucleic acid amplification testing, most commonly via CMV DNA PCR. For decades, the only clinical test of CMV-specific immunity has been serology, which has served as a backbone for pre-transplant immunologic screening of organ donors and transplant candidates. Recently, measures of CMV-specific cell-mediated immunity (CMV-CMI) have emerged from research laboratories to clinical settings. In this article, the author reviews the data on CMV-CMI and provides perspectives on the roles of these assays in the management of CMV in SOT recipients.

2. CMV-CMI Assays: Brief Overview

CMV-CMI assays are laboratory tests that measure activated T lymphocytes after ex vivo stimulation with CMV antigens. Most commonly, activated T lymphocytes are indicated by their ability to express or secrete cytokines, most commonly interferon gamma, after stimulation ex vivo with CMV antigens, most commonly pp65 and IE-1 antigens [10]. There are several CMV-CMI assay platforms that are available from commercial and research laboratories, including an interferon gamma release assay (IGRA; QuantiFERON-CMV [Qiagen, Inc., Hilden, Germany]) [11–15] and quantitation of interferon gamma-expressing cells per pre-determined number of peripheral blood mononuclear cells (PBMC), either through enzyme-linked immunosorbent spot assays (ELISPOT; e.g., T-SPOT.CMV [Oxford Diagnostics, Abingdon, Oxfordshire, United Kingdom] [12], T-Track-CMV [Lophius Biosciences, Regensburg, Germany]) [16,17] or intracellular cytokine

staining through flow cytometry (e.g., CMV inSIGHT T cell immunity testing [Viracor Eurofins, Lenexa, Kansas] [18,19], CMV CD8+ T cell immune competence assay [Mayo Clinic Laboratories, Rochester, MInnesota] [20]). There are other laboratory-developed tests (LDT) that utilize these similar principles of measuring T lymphocyte activation ex vivo in response to CMV antigens [21].

CMV-CMI can measure CD4+ and/or CD8+ T lymphocytes using whole blood or isolated PBMC samples. After collection, blood samples are incubated for a defined period of time (e.g., overnight incubation) in the presence of CMV-specific peptides [19]. Thereafter, the amount of secreted interferon gamma in plasma (QuantiFERON-CMV) [5,11,13,15] or the number of interferon-producing cells (T-SPOT.CMV, T-Track-CMV, flow cytometry) are measured [12,16,19,20]. The results of the CMV-CMI assays are interpreted in the presence of a negative and a positive (mitogen) control; these are included to ensure quality of CMV-CMI testing.

However, there is lack of standardization across the different CMV-CMI assays. The variability in performance among the CMV-CMI assays may be related to differences in their methods, antigenic stimulants, clinical samples, and reporting parameters, among others. Furthermore, clinically available CMV-CMI assays do not fully reflect the full complement of CMV immunity. Most assays measure mainly activated CD8+ T lymphocytes, with interferon gamma production only as the read-out. Accordingly, other components of the CMV immune response, such as NK cells, are not accounted for. In vivo, all immune components are anticipated to synergistically act to control CMV; hence, the functional assessment of only one component (i.e., CD8+) does not fully reflect the global state of CMV-specific immunologic function.

3. Clinical Applications of CMV-CMI Assays

Detection of high levels of interferon gamma in plasma or high numbers of interferon gamma-producing cells (via ELISpot or flow cytometry) generally correlates with CMV-specific immunity and phenotypically, confering protection from CMV infection. Conversely, low levels or absence of these immune measures have been consistently associated with higher risk of CMV disease after SOT. With this general principle, Table 1 lists the potential clinical applications of CMV-CMI assays in the field of SOT, from the pre-transplant to the post-transplant period. The vast majority of clinical studies on CMV-CMI in SOT support its role as a prognosticator for CMV risk. Recently, there have been efforts to utilize CMV-CMI assays to guide decisions on antiviral prophylaxis, preemptive therapy, and treatment of disease. In a series of five questions, the author reviews the evidence supporting the use of CMV-CMI in the clinical setting.

Table 1. Clinical uses of Cytomegalovirus-Specific Cell-Mediated Immunity (CMV-CMI) assays in solid organ transplant recipients.

Clinical Scenario for Use of CMV-CMI Assays		Proposed Guidance
Pre-transplant CMV-CMI to assess CMV risk post-transplant	CMV R−	CMV-CMI is not a useful tool. Almost all CMV-seronegative individuals have negative CMV-CMI, so pre-transplant testing has no prognostic role beyond what is provided by serology.
	CMV R+	CMV-CMI may be a useful tool for post-transplant CMV risk prediction in CMV R+ solid organ transplant candidates. A negative pre-transplant CMV-CMI portends a higher risk of post-transplant CMV infection. A positive pre-transplant CMV-CMI is associated with a lower risk of post-transplant CMV infection.

Table 1. *Cont.*

Clinical Scenario for Use of CMV-CMI Assays		Proposed Guidance
Post-transplant testing to assess CMV risk after transplantation, and guide CMV prevention strategy	CMV D+/R−	CMV-CMI can predict post-transplant risk in CMV D+/R− solid organ transplant recipients, but it is not cost-beneficial or practical when used during antiviral prophylaxis. Almost all CMV D+/R− solid organ transplant recipients have negative CMV-CMI during the period of antiviral prophylaxis. CMV-CMI may not be used to guide the duration of antiviral prophylaxis in CMV D+/R− solid organ transplant recipients.
	CMV R+	CMV-CMI may be useful for post-transplant CMV risk assessment in CMV R+ solid organ transplant recipients. A negative CMV-CMI is associated with a higher risk of CMV, while a positive CMV-CMI is associated with a lower risk of CMV infection. Serial CMV-CMI may be considered for individualizing the duration of CMV prophylaxis in CMV R+ solid organ transplant recipients. Antiviral prophylaxis may be stopped once a robust CMV-CMI is detected. CMV-CMI may be useful for determining the need for preemptive antiviral therapy of asymptomatic low-level CMV DNAemia. A robust CMV-CMI in a patient with asymptomatic low-level CMV DNAemia may lead to self-resolving infection.
Post-transplant guidance of duration of treatment for CMV infection and the need for secondary prophylaxis	CMV D+/R−	CMV-CMI may be a useful tool for post-transplant guidance of CMV treatment, as the development of robust CMV-CMI in CMV D+/R− transplant recipients implies effective immunity and suggests that treatment may be safely discontinued with a low risk of relapse or recurrence. Absence of CMV-CMI at end of treatment (viremia clearance) is correlated with CMV recurrence and suggests the need to consider secondary prophylaxis (and optimization of immunosuppression).
	CMV R+	CMV-CMI may be a useful tool for post-transplant guidance of CMV treatment, as the detection of robust CMV-CMI implies an effective immune reconstitution and safety in stopping antiviral treatment without the need for secondary antiviral prophylaxis. In selected CMV R+ solid organ transplant patients (e.g., highly immunosuppressed), CMV-CMI may be a useful tool for post-treatment guidance of the risk of CMV relapse (and the need for secondary prophylaxis).

Supportive evidence for this proposed guidance is discussed in the body of this article.

3.1. Can I Use Pre-Transplant CMV-CMI to Predict the Risk of CMV After Transplantation?

CMV-IgG serology is the standard pre-transplant test used to determine a transplant candidate's (and donor's) prior CMV exposure and inform the risk after transplant. A negative CMV-IgG serology in a transplant recipient portends a very high risk of CMV disease after transplant if they receive an organ from a CMV IgG-seropositive donor (CMV D+/R− mismatch). In contrast, positive CMV-IgG serology in a transplant candidate correlates with pre-existing CMV immunity and portends a relatively lower risk (but not absolute protection) of CMV infection in the post-transplant period.

Recently, there have been emerging data illustrating that CMV-IgG does not always correlate with the presence of an effective CMV-CMI. In one study of 100 CMV-IgG-positive individuals, there was 18% disagreement between serology and CMV-CMI [22]. In another study of 44 CMV-seropositive lung and kidney transplant candidates, only 30 (68%) had detectable CMV-CMI, as measured by interferon gamma production [23]. In a large study of 583 kidney transplant recipients, about 8% of CMV-seropositive patients had undetectable or low-level CMV-CMI (T-SPOT.CMV) during the pre-transplant period [12].

Moreover, recent studies have demonstrated that absence of CMV-CMI in CMV-seropositive transplant candidates and recipients is significantly correlated with a higher risk of post-transplant CMV infection. In the study of 44 CMV-seropositive lung and kidney recipients, the rate of post-transplant CMV was significantly higher among patients with non-reactive versus reactive pre-transplant CMV-CMI (7 of 14 [50%] vs. 4 of 30 [13.3%],

respectively; $p = 0.021$) [23]. Another study of 30 living donor liver transplant recipients demonstrated that 13 patients with positive pre-transplant CMV-CMI (QuantiFERON-CMV) had lower risk of CMV disease (15.4% vs. 58.8%; $p = 0.016$), faster clearance of viremia (7 days vs. 21 days; $p = 0.004$), and shorter duration of antiviral drug treatment (13 days vs. 28 days; $p = 0.003$) when compared to CMV-seropositive liver recipients with negative pre-transplant CMV-CMI [24]. In a prospective interventional clinical trial of 160 CMV-seropositive patients, those with undetectable or low pre-transplant CMV-CMI had significantly higher rates of post-transplant CMV infection [25]. Based on these observations, CMV-CMI may be considered as a supplement in the pre-transplant assessment of CMV-seropositive SOT candidates with the goal of stratifying patients into high or low risk of CMV after transplantation, potentially guiding CMV prevention strategies.

- *CMV-CMI may be considered as a supplement to CMV serology in the pre-transplant assessment of CMV-seropositive SOT candidates to better inform the risk of post-transplant CMV infection*.

In contrast, pre-transplant CMV-CMI may not be indicated in CMV-seronegative SOT candidates, since there is high concordance between CMV IgG-seronegativity and lack of CMV-CMI. In a prospective observational study that included 260 CMV D+/R− patients, only about 5% of CMV-seronegative individuals had any detectable CMV-CMI (T-SPOT.CMV) in pre-transplant specimens [12]. Since almost all CMV-seronegative persons are CMV-CMI non-reactive [11,12], routine assessment of pre-transplant CMV-CMI among CMV-seronegative transplant candidates is neither a practical nor cost-effective strategy.

- *CMV-CMI should not be routinely assessed during the pre-transplant screening of CMV-seronegative transplant candidates*.

3.2. When Can I Measure CMV-CMI After Transplantation to Predict the Risk of CMV Infection After SOT?

Pharmacologic immunosuppression after SOT may entail the use of T lymphocyte-depleting agents (e.g., anti-thymocyte globulin, alemtuzumab) and impair T lymphocyte function (e.g., basiliximab, mycophenolate, tacrolimus, steroids). The overall effect of these drugs is highest during the first 1–3 months after SOT. Accordingly, CMV-CMI measurement during this time period may be useful to inform the risk of CMV in the post-transplant setting, particularly among CMV-seropositive SOT recipients. However, the specific time points for CMV-CMI measurements after transplantation are neither well defined nor standardized.

Some studies have investigated CMV-CMI as early as 2 weeks after SOT, while others perform CMV-CMI testing serially, on a monthly basis, and others at the anticipated end of antiviral prophylaxis. Among CMV-seropositive patients, there is an expected profound abrogation of CMV-CMI during the first 2–4 weeks after SOT. This marked impairment in CMV-CMI is anticipated to occur in the majority of SOT patients, including those who did not receive T cell-depleting induction therapy, especially if the impairment in T cell function is severe. In one study that measured CMV-CMI (T-SPOT.CMV) on day 15 after transplantation [25], there was profound abrogation in total T lymphocyte counts, which was later found to be a predictor of subsequent CMV infection among patients treated with anti-thymocyte globulins [25]. Among the 124 CMV-seropositive kidney transplant recipients, low levels of CMV-specific CD8+ T lymphocytes (<2.0 cells/μL, measured via intracellular cytokine staining) on post-transplant day 15 had greater subsequent risk of CMV events [26]. Notably, another study reported that no patient with preserved (i.e., positive) CMV-CMI subsequently developed CMV disease [27]. Moreover, almost all patients with CMV-CMI controlled CMV reactivation without needing antiviral treatment [27].

Starting at 30 days after SOT, CMV-seropositive patients display progressive, albeit heterogenous, patterns of CMV-specific immune reconstitution [28]. In a study that included 70 CMV-seropositive SOT recipients, there was a steady and constant CMV immune reconstitution starting on day 60 [28]. In a large study that included 277 CMV-seropositive kidney transplant recipients, CMV-CMI (T-SPOT.CMV) was recovered in the majority

of patients by 3 months after transplantation [12]. In another study of 78 CMV R+ and CMV-CMI-positive patients, 59.5% of the patients had recovered CMV-CMI by day 30, and 82.7% had recovered by day 90 [29]. Having lower counts of CMV-specific CD4+ T cells at days 60 and 180 were associated with a higher incidence of late-onset CMV events [26].

- *CMV-CMI measurement during the immediate post-transplant period may inform the risk of subsequent CMV infection among CMV-seropositive SOT recipients. In general, absence of CMV-CMI in CMV-seropositive SOT recipients is a risk factor for subsequent CMV infection. However, the frequency and time points of CMV-CMI measurements are not well defined*.

CMV D+/R− SOT recipients are at highest risk of CMV disease and are generally provided with antiviral prophylaxis for prolonged durations [4]. Failure to develop CMV-CMI (QuantiFERON-CMV) at the end of antiviral prophylaxis correlated with subsequent risk of CMV disease after completion of antiviral prophylaxis. In a cohort of 124 CMV D+/R− SOT recipients, the rate of subsequent CMV disease was 6.4% among those with reactive CMV-CMI, compared to 22.2% among those with non-reactive CMV-CMI [13].

Multiple studies have consistently demonstrated that CMV-CMI does not develop in the vast majority of CMV D+/R− SOT recipients during the period of antiviral prophylaxis. In one small study that included 13 CMV D+/R− SOT recipients, CMV-CMI (ELISPOT) was not attained during antiviral prophylaxis [28]. CMV-CMI (T-SPOT.CMV) at 3 months, when antiviral prophylaxis was discontinued, was undetectable in all 21 CMV D+/R− kidney transplant recipients [30]. Among the 49 CMV D+/R− kidney or pancreas transplant recipients who had CMV-CMI tested during antiviral prophylaxis, the majority (n = 46; 94%) had undetectable CMI (inSIGHT TCIP) [18]. In a cohort of 24 CMV D+/R− SOT recipients who received 6 months of valganciclovir, only 1 patient (4.2%) demonstrated CMV-CMI (QuantiFERON-CMV) after completion of valganciclovir prophylaxis [11]. In a study that evaluated CMV-CMI (T-SPOT.CMV) in a large cohort that included 257 CMV D+/R− kidney transplant recipients, very few developed CMV-CMI by the end of either 3 months or 6 months of antiviral prophylaxis [12]. Indeed, CMV-CMI was detected only after they have developed preceding post-prophylaxis CMV viremia [31]. Accordingly, CMV-CMI measurement is not routinely recommended during antiviral prophylaxis in CMV D+/R− SOT recipients.

- *CMV-CMI remains non-reactive (or negative) in the vast majority of CMV D+/R− SOT recipients during the period of antiviral prophylaxis. Thus, CMV-CMI measurement is not recommended among CMV D+/R− SOT recipients during the period of, and at the end of, antiviral prophylaxis*.

3.3. Can I Use CMV-CMI Measurement to Guide the Duration of Antiviral Prophylaxis?

Antiviral prophylaxis entails the administration of an antiviral drug for a defined period of time after SOT. It has been proposed, however, that the duration of antiviral prophylaxis may be individualized based on risk profile, as indicated by CMV-CMI assay. Ideally, the antiviral drug should be given until CMV immune reconstitution (i.e., reactive CMV-CMI) has occurred.

However, because CMV D+/R− SOT recipients do not generate CMV-CMI during the period of antiviral (valganciclovir) prophylaxis, CMV-CMI may not be a useful test to guide the duration of prophylaxis in these highest-risk patients [32]. In these patients, CMV-specific CD4+ and CD8+ T lymphocyte interferon gamma and polyfunctional responses were only generated after CMV replication has occurred. This observation illustrates the need for viral replication and antigen presentation in order to develop CMV-CMI, and this is not possible during complete viral suppression via valganciclovir prophylaxis. Accordingly, routine serial CMV-CMI measurements are not useful to guide the duration of antiviral prophylaxis among high-risk CMV D+/R− SOT recipients [33].

- *CMV-CMI measurement is not useful to guide the duration of antiviral prophylaxis among CMV D+/R− SOT recipients*.

In contrast, among CMV-seropositive SOT recipients, the profound abrogation of CMV-CMI, especially among those who received induction with T cell-depleting anti-thymocyte globulin, is only transient, and it is no longer evident in most patients 3 months after transplantation [34]. Most studies demonstrate that CMV reconstitution is achieved in the majority of CMV-seropositive SOT recipients by end of prophylaxis at 3 or 6 months (some even earlier), and this was protective from CMV disease. Multivariate binary logistic regression analysis revealed that lack of CMV-CMI at the time of prophylaxis cessation was the only independent correlate predicting CMV infection among CMV-seropositive SOT recipients [34]. In another study of 60 CMV-seropositive lung transplant recipients, poor CMV-CMI (T-SPOT.CMV) at 6 months (when antiviral prophylaxis was discontinued) was significantly correlated with subsequent post-prophylaxis CMV infection [35]. However, even as early as 30 days following SOT, some CMV-seropositive patients already displayed progressive, albeit heterogenous, patterns of CMV-specific immune reconstitution [28]. This raises the question: can CMV-CMI guide the duration of prophylaxis in CMV-seropositive SOT recipients?

In one open-label non-inferiority trial, 150 CMV-seropositive kidney recipients who received anti-thymocyte globulin induction were randomized to receive a standard, fixed duration of antiviral prophylaxis (up to 3 months) or to have the antiviral prophylaxis discontinued earlier upon the detection of CMV-CMI (QuantiFERON-CMV) [36]. With this strategy, CMV-CMI guidance led to earlier discontinuation of antiviral prophylaxis (median, 57 days) in the majority (59.2%) of patients. Despite shorter durations of antiviral prophylaxis, none of the 76 patients in the immuno-guided prophylaxis group developed CMV disease, compared to 2 of 74 patients who received standard 90-day prophylaxis. Because of the shorter duration of prophylaxis, the rate of neutropenia was significantly lower in the immune-guided prophylaxis group (9.2%, compared to 37.8% among those who received a fixed 90-day duration of prophylaxis) [36].

CMV-CMI (QuantiFERON-CMV) was also used to guide the length of antiviral prophylaxis after lung transplantation [14]. In this interventional study, 118 lung transplant recipients at risk of CMV infection (88 were CMV-seropositive and 30 were CMV D+/R−) were randomized to receive either 5 months of a fixed duration of prophylaxis or to have the duration individualized and extended up to 11 months depending on serial CMV-CMI measurements. The incidence of CMV infection in the lung allograft within 18 months after lung transplantation was significantly lower in the CMV-CMI-directed cohort (37% versus 58%, $p = 0.03$). Of the 80 patients who discontinued antiviral prophylaxis after a fixed duration of 5 months, the incidence of CMV DNAemia (>600 copies/mL) was significantly lower in the patients with a reactive versus non-reactive CMV-CMI (13% versus 67%, $p = 0.0003$). Likewise, the incidence of high-degree viral replication (>10,000 copies/mL) was significantly lower in patients with a reactive versus non-reactive CMV-CMI (3% versus 50%, $p < 0.001$). A non-reactive CMV-CMI after 11 months of antiviral prophylaxis was associated with a 25% incidence of CMV DNAemia [14]. In a retrospective study of 263 lung transplant patients, the majority of the 204 CMV-seropositive lung recipients (76%) achieved a reactive CMV-CMI after 5 months and had antiviral prophylaxis discontinued. CMV DNAemia was uncommon among those with reactive CMV-CMI [37].

These observations collectively suggest that reconstitution of CMV-CMI occurs in the majority of CMV-seropositive patients during the course of standard antiviral prophylaxis, and many even prior to the anticipated end date of antiviral prophylaxis. Accordingly, one may consider performing serial CMV-CMI to determine the time to CMV immune reconstitution, when antiviral prophylaxis may be discontinued (Figure 1A).

- *Serial CMV-CMI measurements may be performed during antiviral prophylaxis among CMV-seropositive SOT recipients. Detection of reactive CMV-CMI suggests immune reconstitution that allows for earlier discontinuation of antiviral prophylaxis.*

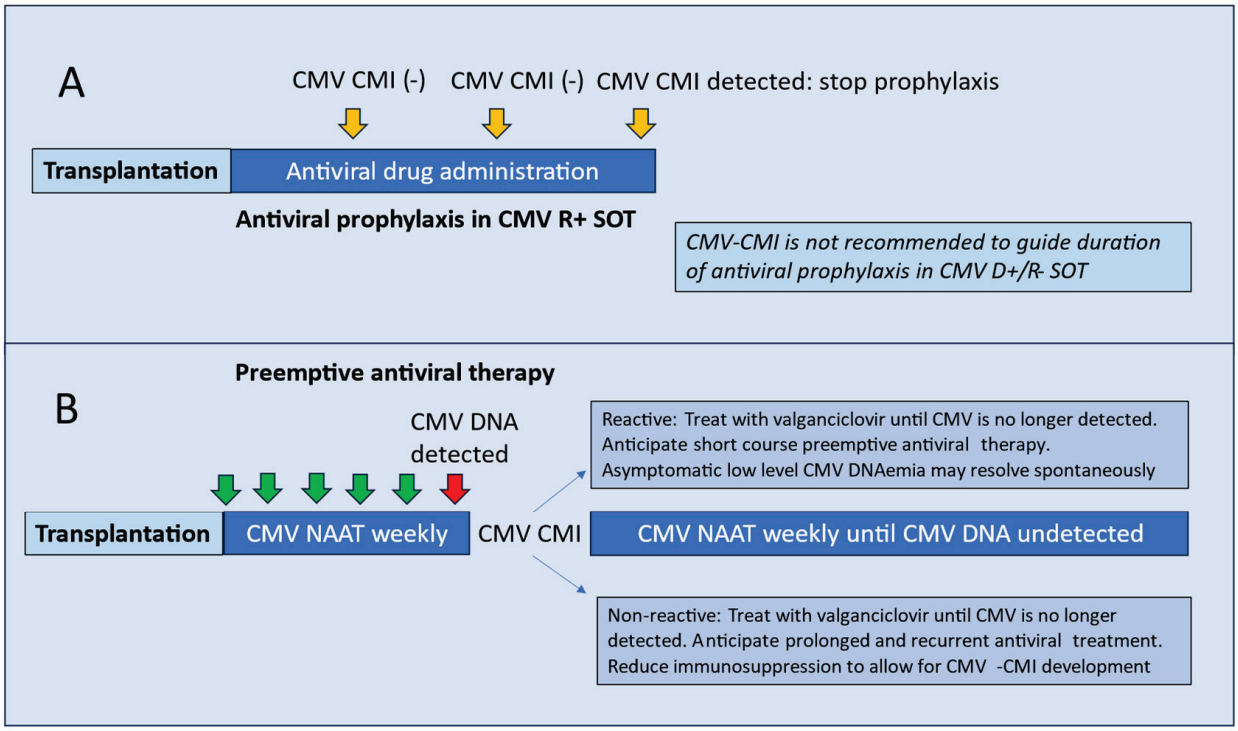

Figure 1. Strategies for cytomegalovirus (CMV) disease prevention and treatment that incorporates CMV-specific cell-mediated immunity. Top panel (**A**)—Antiviral prophylaxis: CMV-specific cell-mediated immunity may be measured during the period and at the anticipated end of prophylaxis among CMV-seropositive solid organ transplant recipients; antiviral prophylaxis may be safely discontinued when CMV-specific cell-mediated immunity is detected in CMV-seropositive solid organ transplant recipients. This approach is not applicable to CMV D+/R− solid organ transplant recipients, since CMV-specific cell-mediated immunity is rarely attained during antiviral prophylaxis. Bottom panel (**B**)—CMV surveillance and preemptive therapy: CMV-specific cell-mediated immunity may be measured at the time of CMV reactivation. CMV-immune patients with low-level viral reactivation may resolve the infection spontaneously or require a short course of preemptive antiviral treatment. CMV non-immune persons are anticipated to need a longer course of antiviral treatment. Footnote: CMV, cytomegalovirus; CMI, cell-mediated immunity; NAAT, nucleic acid amplification test.

Conversely, the absence of CMV-CMI at the anticipated end of standard-duration antiviral prophylaxis (3–6 months) confers a higher risk of CMV infection and warrants the institution of efforts to prevent CMV reactivation. This may be in the form of extended or prolonged prophylaxis, aggressive CMV surveillance post-prophylaxis, and importantly, consideration to minimize the degree of pharmacologic immunosuppression, if feasible, to allow for CMV-CMI reconstitution [34].

3.4. Can I Use CMV-CMI in Guiding the Decision to Treat SOT Recipients with Asymptomatic CMV Reactivation?

Prevention of CMV disease after SOT can be accomplished by preemptively treating asymptomatic CMV infection with oral valganciclovir or intravenous ganciclovir; this is known as a preemptive therapy approach. However, the viral load threshold that triggers antiviral therapy in asymptomatic SOT patients is not known.

Observational studies have reported that asymptomatic CMV reactivation in transplant patients with a robust CMV-CMI are often low-level, transient, and may resolve spontaneously. In one study, CMV-CMI (QuantiFERON-CMV) was measured in 37 SOT recipients with asymptomatic CMV viremia (mean viral load, 1140 copies/mL). Among the 26 patients (70.3%) with a reactive CMV-CMI at the onset of viremia, 24 had spontaneous

clearance of viremia without antiviral therapy [38]. Of interest, in a case series of 12 CMV D+/R− kidney transplant recipients, CMV-CMI (QuantiFERON-CMV) was detected in 6 patients who spontaneously resolved their infection without antiviral therapy [39]. These small-scale studies collectively suggest that assessment of CMV-CMI at the onset of low-grade asymptomatic CMV viremia may inform clinicians of the need to initiate, or withhold, preemptive antiviral treatment (Figure 1B) [38].

- *A robust CMV-CMI at the onset of low-level asymptomatic CMV reactivation suggests the potential for spontaneous viremia clearance that may not require preemptive antiviral drug treatment.*

3.5. Can I Use CMV-CMI to Guide the Duration of Antiviral Treatment of CMV Disease and Inform the Risk of Post-Treatment CMV Relapse?

Intravenous ganciclovir or oral valganciclovir is the standard treatment for CMV infection and disease after SOT. The duration of antiviral treatment is highly individualized and guided by serial CMV NAAT (Figure 2). It is recommended that ganciclovir or valganciclovir treatment is continued until symptoms have resolved and the CMV viral load is undetectable in the blood for 1–2 weeks [4]. Despite this approach, recurrence of CMV infection occurs in about 20% of patients [20,40]. Anecdotal experience suggests that CMV relapse may be associated with an underlying severe degree of immunosuppression. This raises the question: can we use CMV-CMI as a complement to standard viral load testing to guide antiviral treatment of CMV disease?

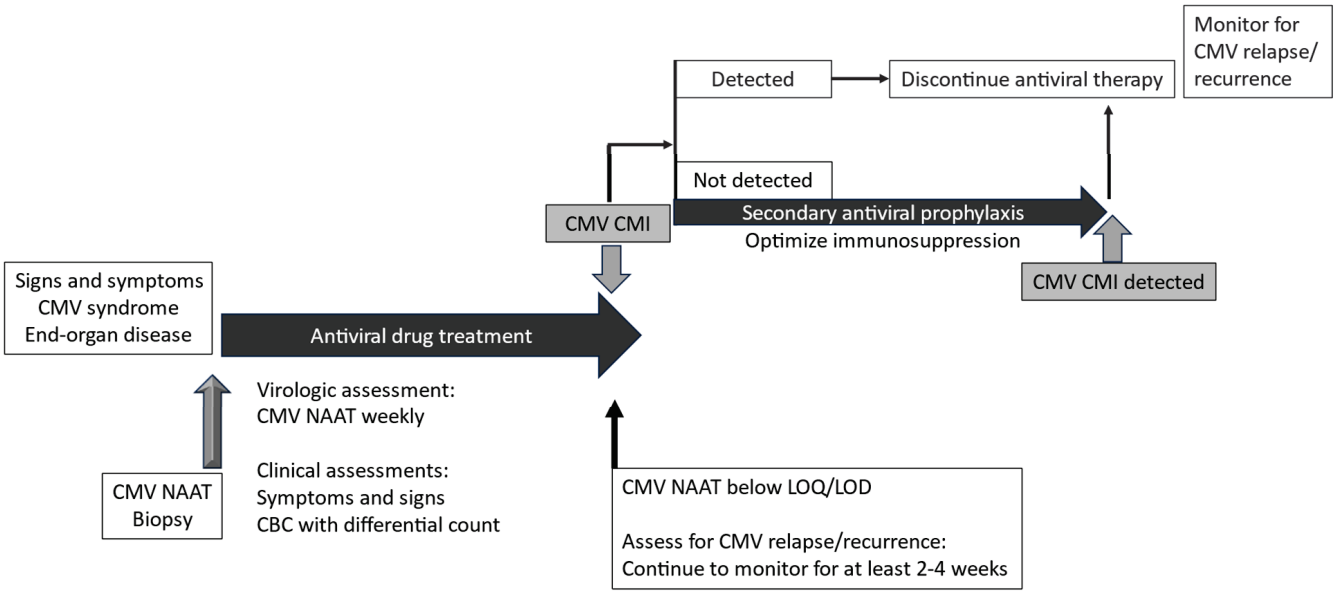

Figure 2. Treatment of CMV infection and disease that incorporates measures of CMV-specific cell-mediated immunity to complement viral load and clinical monitoring to guide the duration of antiviral treatment and the need for secondary antiviral prophylaxis. Footnote: CBC, complete blood count; CMV, cytomegalovirus; CMV-CMI, cytomegalovirus-specific cell-mediated immunity; LOD, limit of detection; LOQ, limit of quantification; NAAT, nucleic acid amplification test.

In one study of 20 SOT patients treated with valganciclovir or IV ganciclovir for CMV infection or disease, none of the 18 patients who developed CMV-CMI (QuantiFERON-CMV) during treatment had relapse after stopping antiviral therapy [11]. In contrast, one of two patients who did not develop CMV-CMI had CMV recurrence [11]. Another study of 17 CMV-seropositive kidney transplant patients with CMV viremia demonstrated that patients with weak CMV-CMI (ELISPOT; <25 SFC/200,000 PBMC) were more likely to progress to CMV syndrome and require longer durations of antiviral treatment [41]. In an interventional study of 27 SOT patients receiving antiviral treatment for CMV infection (me-

dian viral load, 10,900 IU/mL), CMV-CMI (QuantiFERON-CMV) was measured at the time of viremia clearance [42]. Among the 14 patients (51.9%) with reactive CMV-CMI at viremia clearance, antiviral drug treatment was discontinued, and only 1 patient subsequently developed low-level asymptomatic viral recurrence. In contrast, 13 of 27 patients (48.1%) had no detectable CMV-CMI at viremia clearance, and they were provided secondary antiviral prophylaxis for 2 months. Despite secondary prophylaxis, CMV recurrence occurred in nine patients (69%), including one patient who developed UL97-mutant ganciclovir−resistant CMV [42]. This observation suggests that secondary antiviral prophylaxis should be complemented by immune optimization to prevent recurrent CMV infections (Figure 2). In another study of 39 patients who received preemptive antiviral treatment for mostly primary CMV infection, the only variable significantly associated with CMV recurrence after treatment was lack of CMV-CMI (intracellular cytokine staining and flow cytometry) [43]. Finally, among 44 CMV-seropositive heart recipients, viral relapse was associated with the failure to reconstitute CMV-specific immunity (QuantiFERON-CMV) after the resolution of the CMV infection [44].

- *CMV-CMI may be used to complement viral load testing to guide the duration of treatment of CMV disease. Ideally, antiviral treatment should be continued until the virus is undetectable and CMV-CMI is detectable.*

4. Limitations and Future Directions

The major hurdle to the adoption of CMV-CMI in clinical practice at this time is the lack of widespread availability of CMV-CMI assays. For example, QuantiFERON-CMV is not available for clinical use in the United States, although it is used in many centers in Canada, Europe, and Asia. Most CMV-CMI assays are available mainly in specialized referral centers, and many are still within research laboratories. Moreover, many CMV-CMI assays still lack clinical validation and standardization, which may be difficult to overcome due to the many differences in assays, processes, and platforms. It will be very difficult to standardize across all CMV-CMI assays, so there should be efforts to provide clinical validation for each of them. This means defining the optimal timing and frequency of testing for the various SOT risk groups and defining assay-specific thresholds of CMV immune competence.

Current CMV-CMI assays measure only CD4+ and/or CD8+ T cell function via interferon gamma release after ex vivo CMV antigenic stimulation. There are other aspects of pathogen-specific immune response that are not fully accounted for by these CMV-CMI assays. Other immune cells, including NK cells and cytokines other than interferon gamma, are not included in the currently available immune assays. It is possible that a more comprehensive measure of CMV-CMI that incorporates various aspects of the immune response may provide better insight into the CMV–host interactions and improve the clinical utility and interpretation of these assays. Finally, there is a need for more prospective, controlled, and interventional studies to support the promising role of CMV-CMI in CMV management in SOT recipients. Only a few prospective and interventional studies have been performed, although they are needed to support the suggested clinical applications of CMV-CMI, such as (1) pre-transplant prognostication of post-transplant CMV risk in CMV-seropositive transplant candidates, (2) post-transplant monitoring to guide the duration of antiviral prophylaxis in CMV-seropositive solid organ transplant recipients, (3) assessment of the need for preemptive antiviral treatment of asymptomatic CMV reactivation, (4) assessment of the duration of treatment of CMV disease, and (5) assessment of the risk of CMV relapse and the need for secondary antiviral prophylaxis.

5. Conclusions

This review highlights the role of CMV-CMI in the pathogenesis and outcomes of CMV infection after SOT. In general, a deficiency of CMV-CMI has been consistently correlated with an increased risk of post-transplant CMV infection, while highly reactive CMV-CMI is associated with protection from CMV disease. There are several CMV-CMI assays

that are available for clinical use in different regions of the world, including commercial and laboratory-developed tests, but they are not yet standardized and have not been directly compared head-to-head in a controlled and comprehensive manner for the various clinical indications. Accordingly, there is no preferred CMV-CMI assay that can be widely recommended. Instead, one should consider using locally available, clinically validated CMV-CMI assays to predict the risk of post-transplant CMV and thus guide strategies for CMV disease prevention. Emerging data suggest that CMV-CMI can tailor the duration of prophylaxis among CMV-seropositive solid organ transplant recipients, predict the risk of relapse after treatment of CMV infection, and address the potential need for secondary antiviral prophylaxis. However, more interventional studies are needed to further validate the promising roles of CMV-CMI after SOT.

Funding: This research received no external funding.

Conflicts of Interest: The author declares no conflict of interest.

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viruses

MDPI

Review

Arbovirus in Solid Organ Transplants: A Narrative Review of the Literature

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Abstract: The incidence of arbovirus infections has increased in recent decades. Other than dengue, chikungunya, and West Nile viruses, the data on arbovirus in solid organ transplant (SOT) are limited to case reports, and infections in renal transplant recipients account for most of the reported cases. Dengue and West Nile infections seem to be more severe with higher mortality in SOT patients than in the general population. Acute kidney injury is more frequent in patients with dengue and chikungunya although persistent arthralgia with the latter is less frequent. There is no clear relationship between arboviral infection and acute cellular rejection. Pre-transplant screening of donors should be implemented during increased arboviral activity but, despite donor screening and negative donor nucleic acid amplification test (NAT), donor derived infection can occur. NAT may be transiently positive. IgM tests lack specificity, and neutralizing antibody assays are more specific but not readily available. Other tests, such as immunohistochemistry, antigen tests, PCR, metagenomic assays, and viral culture, can also be performed. There are a few vaccines available against some arboviruses, but live vaccines should be avoided. Treatment is largely supportive. More data on arboviral infection in SOT are needed to understand its epidemiology and clinical course.

Keywords: arbovirus; chikungunga; dengue; donor derived; Japanese encephalitis; Powassan; yellow fever; tick borne encephalitis virus; West Nile; zika

1. Introduction

Arboviruses are a heterogenous group of RNA viruses that are maintained in nature in various vertebrate hosts via transmission by arthropods. These viruses generally belong to *Togaviridae*, *Flaviviridae*, *Bunyaviridae*, *Reoviridae*, and *Orthomyxoviridae* family of viruses. A small number of them are known to cause human infections (Table 1). In recent decades, arboviruses like dengue, chikungunya, zika, West Nile, and oropouche virus have re-emerged and spread intercontinentally and established new ecological niches causing widespread human infection in areas not known to be endemic for these infections as a result of human activities as well as vector expansion, possibly related to climate change [1–4]. In addition, organ transplantation is being performed with increasing frequency in low- and middle-income countries, some of which are sites of frequent arobiviral infection. It is estimated that 3.9 billion people live in areas where dengue, yellow fever (YF), zika, and chikungunya virus transmission has occurred [5]. Human infections can be sporadic or occur in outbreaks depending on the vector activity and human interaction with the vector. When introduced into new environments where appropriate vectors exist, these viruses can become established and be transmitted autochthonously. This raises the specter of further spread of these viruses into new territories afflicting the human population. There are limited data on arbovirus infections in solid organ transplant (SOT) recipients [6,7]. In this review we explore the epidemiology, clinical features, diagnosis,

pre-transplant screening, prevention, and treatment of clinically significant arboviruses in SOT recipients (For methods see Supplementary Material S1).

Table 1. Medically significant arboviruses.

***Togaviridae* family:**
Eastern equine encephalitis virus
Western equine encephalitis virus
Venezuelan equine encephalitis virus
Chikungunya virus
Sindbis virus
Ross river virus
Barmah Forest virus
Mayaro virus
***Flaviviridae* family:**
Dengue virus
Japanese encephalitis virus
Murray valley fever virus
St Louis encephalitis virus
West Nile virus
Powassan virus
Tick Borne encephalitis virus
Louping ill virus
Omsk hemorrhagic fever virus
Alkhurma Hemorrhagic Fever Virus
Kyasanur Forest Disease Virus
Zika virus
Yellow fever virus
Usutu virus
Rocio encephalitis virus
***Bunyaviridae* family (Bunyavirales)**
California encephalitis virus
-La Crosse encephalitis virus
-Jamestown Canyon
Rift Valley fever virus
Crimean Congo hemorraghic fever virus
Severe fever with thrombocytopenia syndrome
Heart land virus
Oropouche Virus
Toscana Virus
Cache Valley fever
Ngari Virus
***Reoviridae* family**
Coltivirus
-Colorado tick fever virus
-Seadornavirus (Banna virus)
***Orthomyxoviridae* family**
Thogotovirus genus including thogoto, bourbon and dhori viruses

2. Medically Significant Arboviruses in SOT

2.1. Dengue

Dengue infection is caused by one of the four serotypes of dengue virus (DENV), a flavivirus, which is endemic in tropical and subtropical countries. In recent years autochthonous transmission of dengue has been witnessed in previously unaffected areas including continental United States and Europe [1,8–10]. Worldwide there has been a 10-fold increase in dengue infection from 505,430 cases in 2000 to 5.2 million in 2019 [11]. Since most infections are asymptomatic, the actual annual number of dengue cases is estimated to be much higher, around 400 million [12]. Moreover, half of the world population is at risk of receiving a dengue infection. This puts SOT recipients living or traveling to endemic areas at risk of acquiring dengue infection [13].

2.1.1. Epidemiology

DENV is usually transmitted by *Aedes aegypti* and *Aedes albopictus* species mosquitoes. Other modes of acquisition include blood transfusion, transmission via hematopoietic cell and organ donation, mucocutaneous exposure including needle stick injury, intrapartum/perinatal transmission, and possibly via breast feeding [14–16].

Most cases of dengue infection in SOT have been reported in kidney transplant (KT) recipients [17–24]. Dengue in SOT can occur year-round in endemic areas and any time after transplant [25,26]. In a retrospective study in Brazil, there were only two (0.1%) cases of Dengue among 1754 liver and KT recipients between 2001 and 2006 [18]. The incidence, however, may, of course, dramatically increase during outbreaks [27,28]. Although currently available dengue vaccination is not recommended in SOT, its implementation in routine immunization in high transmission settings is predicted to lower the incidence of dengue infection (especially DENV1 and DENV2 serotypes) in the general population and perhaps in SOT as well [29]. The true incidence of dengue in SOT is not known since almost all dengue literature in SOT are retrospective in nature that included only patients that sought medical care. It is not clear whether SOT recipients are more susceptible to dengue infection compared to the general population but dengue incidence in SOT parallels the trend in the community [30].

2.1.2. Clinical Features

It is estimated that 75% of dengue infections worldwide are clinically inapparent or minimally symptomatic [12]. In the general population, the first episode of dengue among symptomatic patients includes fever, headache, retro-orbital pain, myalgia, arthralgia, nausea, emesis, and rash. Infection by one of the four serotypes does not protect against subsequent infection by the others. Subsequent infections can be severe and can be complicated by shock and hemorrhage in both the general and SOT population [27,31]. In a study of 102 KT recipients with dengue, severe symptoms including shock and hemorrhage were numerically more frequent in secondary vs. primary infection (16% vs. 7%) [27]. However, in the SOT literature, primary and secondary infections are often not differentiated and in some studies they are presumed to be primary or secondary without serological confirmation of prior infection [17,20,21,30,32–35].

In general, clinical features of dengue infection in SOT are similar to those of the general population. In a review of 168 KT recipients with dengue, fever (86% vs. 99%), headache (35% vs. 96 %), myalgia (47% vs. 92%), and arthralgia (20% vs. 76%) were found to less common, whereas pleural effusion (17% vs. 2%) and ascites (35% vs. 1%) were more common compared to a historical cohort of general population [26]. In another study of KT recipients, retro-orbital pain, conjunctival redness, thrombocytopenia on admission, and absence of arthralgia were more frequently encountered in patients with dengue [28]. Pleural effusion and ascites have been reported in several studies in SOT [17,27,32,33,35–38]. Unusual syndromes like colitis, myocarditis, pericardia effusion, cholecystitis, and hemophagocytic lymphohistiocytosis syndrome have also been described [35,39,40]. Bleeding at allograft sites complicated by cardiac tamponade and

graft nephrectomy have been reported in severe cases [22,32,36,41,42]. Arthralgia, however, seems to be distinctly uncommon in SOT, perhaps due to immunosuppressive effect of antirejection medications [25,30,35,38,43].

Cytomegalovirus coinfection has been rarely reported [27,30,34,44]. Other coinfections can also occur [27,28,37,45]. In the study by Nazim et al., bacterial coinfections (including bacteremia, pneumonia, urinary tract infection, lung abscess) occurred in 17%, malaria in 5%, and fungal in 3% of KT recipients [27]. Patients with bacteremia were more likely to have longer hospitalization, severe disease, and death. In fact, in all seven patients who died, death was attributed to bacterial infection.

Thrombocytopenia, leukopenia, and transaminitis are commonly encountered in SOT with dengue infection [25,33,35,37,38,46]. The duration of thrombocytopenia seems to be more prolonged in SOT patients when compared to the general population [27,37,38,47]. In one small study, thrombocytopenia recovery seemed to correlate with serological response [46]. Antimitotic drugs liked mycophenolate used for the prevention of rejection can cause cytopenia but in the study by Nasim et al. antimitotic agents had no effect on the duration and severity of thrombocytopenia [27].

Transient acute kidney injury (AKI) is common, occurring in more than 50% cases in most studies [25,26,28,31,34,35,38,43]. AKI is more frequently encountered in KT recipients compared to non-transplant patients with baseline normal renal function [47]. However, most of them seem to recover renal function and renal allograft failure is uncommon [30,35,47]. In the largest study of dengue in KT recipients, renal allograft failure occurred in only 6.5% patients [26]. Renal function is less likely to recover to baseline in severe dengue [27]. Acute cellular rejection is uncommon [22,31,34,41]. One study from India, however, reported 2 (6%) cases of acute cellular rejection among 31 dengue patients [28].

Some studies have reported an overall favorable outcome with no mortality, [25,28,31,35,44,46] but severe dengue, sometimes fatal, is not uncommon [27,30,33,34,38,43]. In the study by Ribeiro et al., 2 (11%) out of 19 KT recipients, and in another study from South America, 7 (35%) of 20 SOT, developed severe dengue but without reported deaths [31,35]. Although overall mortality is generally less than 10%, it seems to be higher than that of general population with dengue infection [27,30,43]. In a systematic review of 168 KT recipients, severe disease and mortality rates (16% vs. 4% and 9% vs. 0.06%, respectively) appeared to be higher compared to a historical control of the general population [26]. Disease severity and mortality are not associated with gender or time since transplant, although mortality appears to be higher in the early post operative period [26]. The type of immunosuppression does not seem to affect mortality except perhaps cyclosporine containing regimens that seem to be protective against severe dengue in secondary cases [27,43]. New bleeding complications and ascites seem to be associated with the disease severity, graft loss, and mortality [26].

2.1.3. Donor Derived Infection (DDI)

Possible DDIs have been reported in kidney, liver, and heart transplant recipients [19, 21–24,28,32,36,45,46,48]. In some of these reports the evidence is largely circumstantial [21, 22,24,28,32,36,45]. These studies also do not exclude the possibility of dengue transmission via blood product transfusion.

The strongest evidence of DDI was presented by Mathew et al. in a liver transplant recipient where there was near complete nucleotide and amino acid homology in the envelope region of donor and recipient DENV type1 [49]. However, since the same strain of the virus might be circulating in the community, it is difficult to establish DDI definitively even when there is genetic identity between the donor and recipient DENV. Moreover, the recipient's pre-transplant blood was not available so absence of subclinical infection prior to the transplant could not demonstrated.

Donor transmission from aviremic donors has been reported in KT recipients, presumably via contaminated urine in which DENV may be recoverable for longer than in

the blood [46,48]. In one study, two KT recipients from the same donor who had negative blood DENV PCR and NS1 antigen, developed DENV2 infection soon after transplant [48]. Both recipients had negative pre-transplant blood PCR and or serology. The donor's urine, on retrospective analysis, was found to be positive for DENV2 by PCR but it could not be analyzed by further sequencing. However, genetic sequencing of DENV isolated from the donor's husband, who had dengue infection around the same time as the donor, matched that of the recipients. The recipient of a liver from the same donor did not develop dengue, suggesting urine was the probable route of DENV transmission.

Detection of DENV by PCR in podocytes in a KT recipient suggests that the virus can persist and possibly replicate in the kidney and can serve as a transmission source [50]. In the general population, DENV PCR in the urine may remain positive longer than in the serum, consistent with transmission via the urine and urinary tract being a potential route of transmission to KT recipients, but detection of viral RNA does not necessarily indicate the presence of replicable virions. The incidence of detection of DENV by PCR in the serum is >50% on day 0–7 of infection but then decreases significantly, whereas in the urine the rate of positivity is >50% on days 6–16 [51]. Moreover, DENV has been detected by PCR and immunohistochemistry but rarely by culture in various organs including kidney, liver, and lung from fatal cases of dengue [17,52].

DDI usually occurs within the first 10 days after transplantation. The outcome of early infection is similar to that after later onset. The symptoms range from none but with laboratory abnormalities (some diagnosed during investigation of dengue in other recipients from the same donor) to severe and even fatal [22,32,36,45,46,48]. In contrast, in a review of DDI in liver transplantation, five out of six transplant recipients had severe dengue and two died [24].

2.1.4. Diagnosis

The diagnosis can be confirmed by blood PCR and or NS1 antigen detection. Blood PCR and NS1 are generally positive in the first week after illness onset but sensitivity decreases considerably thereafter in the normal host [51]. However, in the immunocompromised host, blood and more specifically urine PCR can remain positive for weeks [45,46,48,50]. In one study, blood and urinary PCR remained positive up to 65 and 365 days, respectively [46]. Positive PCR does not necessarily equate with the presence of infectious virions and urinary viral isolation by culture is generally positive for only a few days [36,48]. However, in one report of a lymphocytopenic KT recipient, urinary DENV was isolated for as long as 9 months after infection suggesting persistent viral replication [50]. Clearance of viruria or urinary PCR seems to coincide with CD8T cell and or general lymphocyte recovery [46,50].

Detection of the IgM antibody against DENV is less specific due to cross reactivity with other viruses, but it remains positive longer in the normal host. Serum IgM is usually first detected a week after onset of illness. In SOT, serological response can be suboptimal or delayed [27,46,49]. In most studies of dengue in SOT, serum IgM and NS1 have been employed more often than PCR [25,34]. Ideally combination of serum DENV PCR, NS1, and serum IgM will maximize sensitivity. In one study serum PCR, NST1, and IgM were positive in 87%, 72%, and 84% of cases [34]. A positive IgM alone should be interpreted in a clinical context. When possible, a positive IgM should be confirmed by a neutralizing antibody using quantitative assays like plaque reduction neutralizing tests (PRNT). While PRNTs are less likely to be false positive compared to IgM tests, their specificity decreases in areas endemic for multiple flaviviruses. Infection with one flavivirus can induce multiple neutralizing antibodies against other flaviviruses too (especially in seconday infection), and this can hamper the identification of the infecting virus [53]. Ideally serology should be performed on both acute and convalescent samples to confirm recent infection.

2.1.5. Pre-Transplant Screening

Pre-transplant screening for dengue in asymptomatic donors and recipients is controversial. Some centers in endemic areas have implemented routine donor and or recipient screening by blood PCR (and urine PCR among KT donors) [30,46,48]. In Singapore, a dengue endemic country, universal blood dengue PCR screening in deceased donors was implemented in 2016, and in 2021 urine PCR was added to the universal screening protocol [54]. Among 207 deceased donors screened between 2016 and 2022 after universal blood PCR was implemented, only 1 (0.5%) donor tested positive. Although no cases of DDI were recorded after universal donor screening was initiated, the positivity rate among screened deceased donors was exceedingly low. Some authors recommend donor screening only during outbreaks [31,55]. However, positive dengue tests in deceased donors, unlike SOT recipients, may not corelate with community outbreaks [30,54]. South American guidelines recommend screening donors for exposure risk and suggest blood PCR screening in areas with ongoing viral activity [56], and South Asian guidelines recommend donor and recipient screening with NS1 or IgM during increased disease activity in the community [57].

Routine screening in resource limited endemic areas can be difficult to implement, and blood/urine PCR can add to the cost of already overwhelmed resources. Moreover, a negative PCR and NS1 antigen does not rule out recent infection since the sensitivity of both tests decrease after one week of illness, and DENV can be present in various organs with potential for reactivation after the transplant [50]. Moreover, the NS1 antigen has suboptimal sensitivity in secondary dengue infections [58]. Serum IgM can be used in resource limited settings, but it can be a false negative early on and can cross react with other viral infections. It might be prudent for individual transplant centers to establish their own criteria for donor/recipient screening based on disease prevalence in the community and available resources. However, if a donor is known to have dengue within last 30 days, transplantation should be avoided [56,57].

2.1.6. Prevention

Avoidance of mosquito bites using protective clothing, insect repellants, and mosquito nets (day time sleeping) is likely the most cost-effective preventive strategy. Integrated pest management that includes using larvicides and adulticides and reducing mosquito habitats is needed to control mosquitoes [59]. Although not yet recommended by the World Health Organization (WHO), deployment of laboratory infected *A. aegypti* mosquitoes by *Wolbachia*, an intracellular bacterium, seems to be promising in endemic areas to control dengue infection [60]. These infected mosquitoes have a reduced ability to reproduce and are less likely to carry and transmit arboviruses including DENV.

Two live attenuated tetravalent dengue vaccines have been approved by the WHO in dengue endemic areas. CYD-TDV (Dengvaxia) is no longer manufactured due to lack of demand and difficulty implementation in immunization protocols in endemic areas (since it required screening for evidence of prior dengue infection). It had previously been recommended for people aged 9–45 years by the WHO (6–16 years in dengue endemic United States territories) who had laboratory confirmation of prior dengue infection. TAK-003 (Qdenga) is the only available vaccine approved by the WHO (but not approved in the United States) for patients 6–16 years of age (and for all persons $\geq$ 4 years of age in Europe) during high dengue transmission settings. Unlike CYD-TDV, TAK-003 can be used even in dengue seronegative people and, hence, does not require screening for prior dengue infection, but its efficacy against DENV3 and DENV4 in seronegative individuals is unproven, and there is some concern of severe infection among those without prior infection if infected with a virus of these serogroups [29]. TAK-003 is a live attenuated vaccine and not recommended in SOT. It can, however, be considered in SOT candidates prior to transplant in endemic areas with high rates of dengue transmission, and certain at-risk travelers to endemic areas, especially those who are likely to have had prior dengue and at risk of having a severe dengue infection during travel [29,61].

2.1.7. Treatment

Treatment is supportive. Although the type of immunosuppressants do not seem to affect the overall outcome in most studies, immunosuppressive medications have been decreased or modified, partly due to cytopenia [25,30,34,35,38]. There are a few reports of successful liver transplantation in immunocompetent patients with dengue related acute liver failure [62,63]. Dengue is, however, a systemic infection, and there is a concern that the infection might worsen after immunosuppression, and liver transplantation is not routinely recommended for dengue-related acute liver failure [64,65].

2.2. Japanese Encephalitis (JE)

JE, caused by JE virus (JEV), is endemic in Southeast Asia and the Western Pacific, especially in rural and agricultural areas. It is generally transmitted by Culex species mosquitoes. Most infected people are asymptomatic or have mild symptoms. Only less than 1% develop encephalitis with significant mortality and morbidity.

2.2.1. Epidemiology, Clinical Features, and Diagnosis

JE has been rarely described in SOT and, thus, its clinical course in this population is not well known [66,67]. In one case, JEV was transmitted to a lung transplant recipient via blood product transfusion with a fatal outcome, while another immunocompromised patient who also received blood products from the same donor had only asymptomatic seroconversion [66]. Serum and cerebrospinal fluid (CSF) JEV IgM testing are the primary modes of diagnosis, but SOT patients may not be able to mount a serological response and, in addition, the commercially available JEV IgM test is non-specific and can cross react with other viruses. Confirmatory plaque reduction neutralization tests (PRNT) may be available in reference centers. Serum JEV PCR in the general population with suspected infection has poor sensitivity due to low grade, transient RNAemia [68]. However, it might be a useful adjunctive diagnostic tool in SOT as was seen in the fatal case of JE in a liver transplant recipient where JEV RNA was detected in serum, CSF, and bronchoalveolar lavage [66].

2.2.2. Pre-Transplant Screening

Routine donor and recipient screening is not recommended in endemic areas due to the rarity of this infection in SOT as well as the possibility of having false positive screening test results. However, it can be considered during outbreaks [57]. Suboptimal sensitivity of PCR and cross reactivity of serology makes screening challenging.

2.2.3. Prevention

Mosquito control: Integrated mosquito management programs (e.g., mosquito surveillance, reduction in mosquito breeding sites, use of larvicides and adulticides, community education, etc.) and avoidance of mosquito bites using protective clothing, insect repellants, and impregnated mosquito nets at night time are the most cost-effective preventive strategies.

In the United States, the inactivated Vero cell culture-derived JE vaccine is available for travelers (including SOT recipients) to endemic areas who are at increased risk of becoming exposed to JEV. Although its efficacy in SOT is not clear, the seroprotection rate in adults after the standard two-dose vaccination is generally greater or equal to >95%. Other types of JE vaccines including live attenuated, live recombinant, and mouse brain-derived vaccine are available in other parts of the world and are recommended to the at-risk general population. However, live vaccines should be avoided in SOT recipients.

2.2.4. Treatment

Treatment is supportive.

2.3. Chikungunya

Chikungunya is a viral infection caused by Chikungunya virus (CHKV), and like DENV it is transmitted by *A. aegypti* and *A. albopictus* mosquitoes. Other modes of ac-

quisition include transmission via blood products and intrapartum transmission. In the last two decades, CHKV has spread to newer areas of the world and has the potential to establish autochthonous transmission in areas where *A. aegypti* and *A. albopictus* are already present [69].

2.3.1. Epidemiology

Its predilection and incidence in SOT are not known, and the latter is affected by its background prevalence and the occurrence of outbreaks. Nonetheless, it seems relatively rare in SOT, probably due to underreporting and since most cases of chikungunya have occurred in places where organ transplantation is relatively infrequent. Chikungunya in SOT was first mentioned in 2007 in three KT recipients among 610 patients who had atypical clinical features of chikungunya during an outbreak in the reunion island [70]. Since then, there have been scattered reports of chikungunya in SOT, predominantly in kidney and liver transplant recipients [71–79].

2.3.2. Clinical Features

Unlike dengue, most infected patients in the general population are symptomatic and present with a sudden onset of fever, headache, myalgia, cutaneous rash and arthralgia, and/or arthritis after an incubation period of 2–12 days [69]. Chikungunya can occur any time after a transplant. In a series of 32 KT recipients from Brazil, the mean time from transplant was 27.5 months (2–307 months) [77]. Most clinical features were like that of the general population with fever reported in 88%, rash in 47%, conjunctival hyperemia in 28%, and headache in 75%. All patients had arthralgia, and almost half of them had arthritis. Arthralgia is usually symmetrical and involves multiple joints—predominately small and medium joints [73,77]. Compared to the general population, musculoskeletal pain, including arthralgia and arthritis, seems to be less common in SOT [73,77,79]. Atypical and severe features like pneumonitis, myocarditis, and encephalitis are uncommon in SOT [70,71,78]. Transient AKI is not uncommon, occurring in 21% KTRs in one study [73,77,79]. Leukopenia including lymphopenia, thrombocytopenia, and transaminitis can occur [73,75,77,79]. CHKV viral load in the blood can be more than 1 million copies/mL and many SOT patients can have comorbidities that can be associated with severe disease [75]. However, the clinical course in SOT is generally benign except for persistent arthralgia [73–75,77,79]. Hospitalization may be required for AKI and pain management for arthralgia [73,77]. Chikungunya does not seem to be associated with rejection [73,75,79].

2.3.3. DDI

DDI has not been documented in SOT. However, infectious CHKV has been found in eye tissues of infected persons, and the spleen, liver, and muscle in non-human primates, suggesting a possibility of DDI from infected donors [80,81].

2.3.4. Diagnosis

In the general population, serum/plasma PCR is usually positive in the first week of illness, and serum/plasma IgM and neutralizing antibodies are detected in the second week. However, SOT recipients may not be able to mount a serological response, thus, causing difficulties in diagnosis. Viral culture can also be performed in the first days of illness, but it may not be feasible in most settings.

2.3.5. Pre-Transplant Screening

Although some centers in endemic areas may perform universal donor blood chikungunya PCR screening, routine pre-transplant screening of asymptomatic donors and recipients is not recommended (see Section 2.2) [54,56,57]. However, during increased disease activity in the community, plasma/serum PCR screening of donors can be considered (see dengue) [56]. If a donor is known to have chikungunya within the last 30 days, transplan-

tation should be avoided [56,57]. Successful kidney transplantation from a donor after recovery from chikungunya and who subsequently had negative serum PCR has been reported [82].

2.3.6. Prevention

Mosquito control: See dengue prevention (Section 2.1.6).

In November 2023, the first Chikungunya vaccine (Ixchiq) was approved in the United States for at-risk patients, but it is a live attenuated vaccine and not recommended for SOT recipients.

2.3.7. Treatment

Treatment is supportive. Joint pain, which may be persistent after resolution of active infection, can be severe and may require steroids (or escalation of steroids if they are already on) for relief [73,74]. Methotrexate with or with leflunomide in combination with steroids seems to be effective in severe cases [83]. There are no specific data on the management of immunosuppressants during chikungunya infection.

2.4. Yellow Fever (YF)

Yellow fever virus (YFV), the etiologic agent of YF, is endemic in regions of Africa and Central and South America. In its natural habitat (sylvatic cycle), the virus circulates among non-human primates via the Aedes species in Africa and via Haemagogus and Sabethes species in South America. Humans become infected when they encounter these vectors in their natural habitat, and the virus can circulate among humans via the urban vector, *A. aegypti* (urban cycle). The vaccine virus has been documented to be transmitted via blood transfusion and breast milk [16,84]. Case fatality rates among those with severe disease can be 30–60%.

2.4.1. Epidemiology and Clinical Features

There are only a handful published cases of YF in SOT, making it difficult to generalize clinical findings [85–87]. Cases present with non-specific fever, malaise, and gastrointestinal symptoms [85–87]. Neurologic symptoms like unsteady gait, tremors, diplopia, nystagmus, myoclonus, and hemiparesis may develop [87]. Transaminitis and hyperbilirubinemia can be prominent. AKI and thrombocytopenia may develop. In severe cases, progressive encephalopathy and liver failure can occur.

2.4.2. DDI

Transmission of vaccine strain YFV has been reported in four SOT recipients from a common deceased organ donor [87]. The organ donor had received a blood product donated six days after receiving the YF vaccine by a third party. Three days after receiving blood transfusion, organs were procured for transplantation. The four organ recipients (two kidneys, a heart, and a liver) developed symptoms within 6 weeks of transplantation. All of them developed significant neurologic symptoms and two of them eventually died. YFV was detected in all recipients by either PCR, metagenomics, or serology (CSF or serum). YVF detected in the CSF in a KT recipient and brain tissue in heart transplant recipient was identical or similar to the vaccine strain on sequencing. The organ donor, however, tested negative for YFV, likely due to dilutional effect of fluids received during treatment or the presence of only low-level viremia.

2.4.3. Diagnosis

In infection in the general population, serum viral RNA may be detected in the first 4 days of illness. Viral culture (blood) can also be positive early in illness but may not be feasible in most clinical settings. YFV can, however, be detected for several days by culture or RT-PCR in other body fluids like urine and semen during convalescence even when serum PCR is negative [88]. Serum YFV-specific IgM testing can be used later in

illness, but its specificity is limited by cross reactivity with other flaviviruses. Moreover, YF vaccine recipients can have persistently positive serum IgM for several years. Whenever possible, a positive serum YF IgM should be confirmed by a more specific PRNT. In SOT recipients with severe disease, YF-specific IgM and metagenomics have been used in CSF for diagnosis [87]. Real time-PCR and immunohistochemistry in tissue specimens can also be helpful in establishing disseminated disease [86,87,89].

2.4.4. Pre-Transplant Screening

Routine pre-transplant screening is not performed (see Section 2.2). The donors should be screened for recent exposure or travel to endemic areas. During an epidemic, serum YFV PCR screening can be considered [56]. Organs from persons with YF disease and recent YF vaccination should be avoided for 30 days, and blood donation for YF vaccine recipients should be avoided for at least 2 weeks [56,87].

2.4.5. Prevention

Mosquito control against urban YF: See Dengue prevention (Section 2.1.6).

Live attenuated YF vaccine is available for prevention of YF infection. Although (inadvertent) YF vaccination in SOT recipients several months or years after transplant seems to be safe and immunogenic, it is generally contraindicated in this population [90–95]. Vaccinated patients who later undergo SOT can maintain serological immunity even years after transplant despite immunosuppression [96,97]. Hence, whenever possible, at-risk patients should be vaccinated prior to transplantation. Due to an increased risk of YF-associated viscerotropic and neurologic disease, the benefits and risks of vaccination should be weighed carefully when vaccinating patients $\geq$ 60 years.

2.4.6. Treatment

Treatment is supportive. There are anecdotal cases of successful liver transplantation in non-transplant patients with liver failure from YF [89,98,99]. However, YF is a systemic disease affecting various organs, and YFV can infect the engrafted liver [89]. In one report from Brazil, only 6 (20%) of 30 non-transplant patients who underwent liver transplantation for liver failure survived [99]. Hence, liver transplantation cannot be routinely recommended for YF-related liver failure.

2.5. Zika Virus

Zika is a systemic infection caused by Zika virus (ZKV). It is primarily transmitted by *A. aegypti* and *A. albopictus* in endemic areas. Other modes of transmission include perinatal transmission, and transmission via sexual contact and blood products. Transmission via animal bites, needle stick injury and saliva, urine, and breast milk (despite the presence of replicative ZKV RNA in various body fluids) is not well established [16,100].

2.5.1. Epidemiology

ZKV can establish autochthonous transmission in areas with no prior history of zika infection [3,101]. When ZKV spread to South America in 2014, there was widespread apprehension that it would be an especial problem among potential organ donors and recipients [102,103]. Surprisingly there have only been five case reports of zika in SOT to date [104–106]. The first ever case series in SOT described two liver and two KT recipients with zika infection 43–590 days after transplant [105]. The mode of acquisition is not clear. In one report, a liver recipient received ZKV infection via infected platelet transfusion on the day of transplant from a blood donor who developed symptoms three days after blood donation [104]. The recipient remained asymptomatic but replicative viral RNA was detected in the serum, which, on genomic sequencing, matched closely with viral RNA in archived blood of the donor. In another report, a heart transplant recipient developed fatal ZKV infection eight months after the transplant [106].

2.5.2. Clinical Features

In the general population, most of the infections are asymptomatic. Symptomatic patients may develop self-resolving fever, rash, headache, arthralgia, myalgia, and conjunctivitis. Neurologic symptoms like Guillain–Barré syndrome and congenital zika syndrome (especially microcephaly in a newborn) have also been reported.

In the published literature on SOT, the symptoms have ranged from asymptomatic to fatal [104,106]. Fever and myalgia seem to be common, but rash and conjunctivitis were not described in the published cases. In the fatal case of zika infection in a heart transplant recipient, the patient presented with fever, headache, malaise, hemiplegia, and seizure [106]. The patient was eventually found to have meningoencephalitis. MRI of the brain showed hypo- and hyperintense lesions in the cingulate and superior frontal gyrus. CSF analysis showed lymphocytic pleocytosis with elevated protein and positive ZKV PCR in the CSF. Immunosuppression was reduced, but the patient had progressive neurologic decline and developed refractory shock secondary to allograft rejection. On autopsy ZKV was detected in various organs including the brain, heart, liver, and lung by PCR, immunofluorescence, and or electron microscopy. In the case series of four SOT with zika, all were hospitalized and had bacterial co-infection. Two of them had fever and three patients had myalgia. All had thrombocytopenia and the liver recipients developed transaminitis while the KT recipients developed AKI. One liver transplant recipient required re-transplantation three months later due to hepatic artery thrombosis and biliary stenosis, which may or may not be related to ZKV. None had neurologic symptoms and all survived.

2.5.3. DDI

Although ZKV transmission from a blood donor to a liver recipient has been reported, no ZKV transmission from an organ donor has been described [104]. In one study, two kidneys from a donor with positive ZKV serum IgG (but negative serum IgM and negative serum and urine PCR) were transplanted [107]. None of the KT recipients developed zika infection. However, ZKV has been found in various body fluids in infected people. ZKV has also been shown to infect proximal tubular epithelial cells, glomerular podocytes, and endothelial and mesangial cells [108–110]. ZKV RNA is found to persist longer in the urine than serum PCR and replication competent virus has been isolated from urine [111]. Thus, the kidneys can serve as reservoir of ZKV and can potentially transmit infection via a renal allograft.

ZKV can also replicate in human cornea, albeit less efficiently, and ZKV RNA has been found in aqueous and vitreous humor and a conjunctival swab, but there has been no documentation of DDI via a corneal graft [112–114]. In one study a deceased donor with negative serum ZKV RNA was found to have ZKV RNA in the vitreous humor after cornea transplantation in two patients [115]. None of the recipients developed zika infection.

2.5.4. Diagnosis

Serum and urine ZKV PCR can be used for diagnosis in early infection. In the general population, serum PCR is usually positive in the first week after symptom onset and urine PCR remains positive for 2 weeks although a longer duration of RNA in serum and urine has been reported [116]. Whole blood PCR seems to be more sensitive than serum [117]. Viral culture can be performed in early infection but may not be pragmatic. Serum IgM is generally positive after the first week of infection and can persist for several weeks. But IgM can cross-react with other viruses and can be false positive [118]. Whenever possible PRNT should be performed to confirm a positive serum IgM, but PRNT may not be able to confirm whether the current infection is from ZKV or a recently exposed flavivirus like dengue, especially when there is cocirculation of $\geq$2 similar arboviruses in the community [53]. Zika PCR can also be used in other body fluids or organs like the brain, CSF, and aqueous and vitreous humor [106,112–115]. Immunofluorescence and Immunohistochemistry can also be used to detect ZKV in tissues [106].

2.5.5. Pre-Transplant Screening

Routine testing of donors and recipients for ZKV infection is not recommended (see Section 2.2), although some centers in endemic areas may perform universal donor blood PCR screening [54]. During epidemics, routine serum or blood PCR monitoring can be considered [56]. In non-endemic areas, donors (and recipients) who have had possible exposure to ZKV due to travel or sexual contact should be tested for ZKV infection [56,116]. The ideal way to screen for ZKV is not clear since serum/blood PCR is only transiently positive, while a positive serum IgM test result can linger on for months. Organs from asymptomatic donors with negative plasma/blood and or urine PCR but positive serum IgM and IgG can be accepted after weighing the risk–benefit ratio [107,118]. Organs from donors with zika infection should not be accepted for 120 days [57].

2.5.6. Prevention

Mosquito control: See dengue prevention (Section 2.1.6).

SOT recipients should avoid unprotected sex with a male partner potentially exposed to ZKV for 3 months after the return of the male partner from an endemic area or after symptom onset [119]. Similarly, male SOT recipients should avoid unprotected sex with a female partner potentially exposed to ZKV for 2 months after return of the female partner from an endemic area or after symptom onset [119]. Pregnancy should be avoided during this time.

2.5.7. Treatment

Treatment is supportive.

2.6. Powassan Disease

Powassan virus, the etiologic agent of Powassan disease, is endemic in the United States (especially in Northeast and around the Great lakes area), Canada, and Russia. It is transmitted by the bite of infected Ixodes species (especially *I. sacpularis*) and less commonly via blood transfusion.

2.6.1. Epidemiology

Descriptions of Powassan disease in SOT are limited to case reports. One report described Powassan virus transmission in a KT recipient in the immediate post-transplant period via blood transfusion from an asymptomatic donor with history of tick bites [120]. The other case report described Powassan disease, presumably contracted by a tick bite, in a KT recipient 14 years after transplant [121].

2.6.2. Clinical Features

Infection in the general population is mostly asymptomatic. It can, however, cause encephalitis with 10% mortality among those with severe disease and with significant morbidity among survivors. It has a long incubation period of 1–4 weeks. In a SOT population there are very little data on the clinical course of Powassan disease. The initial signs and symptoms can be non-specific fever, headache, myalgia, and diarrhea with progression to neurologic symptoms including encephalitis [120,121]. CSF may show lymphocytic pleocytosis and elevated protein. Brain MRI may show T2 enhancement of the brainstem and the cerebellum. In the two cases described in the literature, both patients survived with residual neurologic deficit [120,121].

2.6.3. Diagnosis

Blood and serum PCR can be positive in the early stage of the disease. PCR can also be performed in the CSF and formalin fixed tissues. A positive serum and CSF IgM test supports the diagnosis, but whenever possible a positive IgM test should be confirmed by a PRNT. Immunohistochemistry can also be performed on fixed tissue specimens.

2.6.4. Pre-Transplant Screening

Routine pre-transplant screening is not recommended (see Section 2.2). Organs and blood products from donors with Powassan disease should be avoided for at least 4 months.

2.6.5. Prevention

In endemic areas, preventive measures should be taken to avoid exposure to ticks. There is no vaccine available against Powassan disease.

2.6.6. Treatment

Treatment is largely supportive. The role of steroids and intravenous immunoglobulin (IVIG) is not clear.

2.7. Tick Borne Encephalitis (TBE) Virus

Tick-borne encephalitis virus (TBEV) is endemic in parts of Europe and Eastern Asia and causes TBE. There are three different stains of TBEV—European, Siberian, and Far Eastern; the latter two have a worse outcome. It is primarily transmitted by Ixodes species and via ingestion of contaminated dairy products. Other modes of transmission include handing of infected material (possibly via aerosolization and possibly via percutaneous and mucosal exposure), slaughtering viremic animals, breast feeding, blood transfusion, and organ transplantation [122,123].

2.7.1. Epidemiology

TBE is uncommon in SOT. In a Swiss study involving 4967 SOTs during 2008–2019, there were only 2 cases of TBE, with an incidence rate of 0.09/1000 person-year [124].

2.7.2. Clinical Features

In the general population, most infected patients are asymptomatic. Among symptomatic patients, the illness can be biphasic with an initial febrile syndrome followed by an asymptomatic period and then progression to neurological symptoms including encephalitis.

Based on limited case reports, TBE can have a worse outcome in SOT. Fatal encephalitis has been reported in transplant recipients [125–127]. The symptoms start with a febrile illness and progress to neurologic symptoms. These patients may have a monophasic illness without an asymptomatic period in between febrile illness and neurologic symptoms [126]. CSF can be normal or demonstrate lymphocytic pleocytosis with elevated protein levels. Brain MRI may show T2 hyperintensity in the brain stem and the cerebellum

2.7.3. DDI

A cluster of fatal DDI was reported in three SOT recipients who received organs from the same donor [126]. The incubation period of 17–49 days in these patients seems to be longer than median incubation period of 8 days in the general population. The donor lived in an endemic area. The genomic sequencing of donor and recipients' viral RNA confirmed the same viral strain.

2.7.4. Pre-Transplant Screening

Routine pre-transplant screening is not recommended (see Section 2.2).

2.7.5. Diagnosis

Blood or serum PCR can be performed in the early stage of the disease. PCR also be conducted on urine, CSF, and brain tissue [125]. Next generation sequencing has also been conducted on the brain tissue and CSF [126]. A positive IgM in the sera and CSF is suggestive of infection but whenever possible should be confirmed by PRNT.

2.7.6. Prevention

In endemic areas, preventive measures should be taken to avoid exposure to ticks and consumption of unpasteurized dairy products. An inactivated vaccine against TBEV is available in the Unites States and is recommended for at-risk travelers to endemic areas [123]. It is also recommended for at-risk laboratory workers. Its effectiveness after a 3-dose series against the European strain is >90%, although the effectiveness can be diminished in SOT recipients. It should be noted that there are other local vaccines available in endemic areas.

2.7.7. Treatment

Treatment is supportive. There is no clear role of IVIG (including TBEV specific IVIG) [123].

2.8. West Nile Virus (WNV)

WNV is transmitted to humans through the bite of Culex mosquitoes and accounts for the majority of mosquito-borne illnesses in the continental United States [128]. Transmission also occurs via blood transfusion, organ transplantation, intrauterine exposure, breast feeding, and percutaneous injury [4,129]. Autochthonous transmission with new areas of endemicity can occur in previously unaffected geographic areas [4].

2.8.1. Epidemiology

WNV infection peaks during increased mosquito activity. During 2009–2018, approximately 90% of reported WNV illnesses in the United States occurred between July and September [130]. SOT recipients are at a higher risk of severe WNV disease [130,131]. Transfusion-transmitted infection can occur after receiving infected blood products directly by the SOT recipients or indirectly from allografts from organ donors who received blood products prior to organ procurement [132–134]. KT recipients account for more than half of WNV infection, likely reflecting a larger volume of kidney transplantation [135].

2.8.2. Clinical Features

In the general population, 80% WNV infections are estimated to be asymptomatic, and most symptomatic patients have mild febrile illness with body aches, headache, gastrointestinal symptoms, and rash. Only <1% develop neuroinvasive disease (encephalitis, meningitis, and acute flaccid paralysis), although its incidence increases with age (0.02 vs. 1.22 cases per 100,000 population among aged < 10 years vs. $\geq$70 years, respectively) [130]. Case fatality rate also increases with age and is higher in those with encephalitis (14%) and acute flaccid paralysis (13%) vs. those with meningitis alone (2%).

Although SOT recipients can have asymptomatic or mild illness, they are at a higher risk of having neuroinvasive disease. During a 2002 WNV outbreak in Canada, the incidence of neuroinvasive WNV was found to be 40 times higher in SOT compared to the general population [131]. The increased risk was probably overestimated due to the small number of infected SOT patients. Nonetheless, in a review of 53 cases of WNV in SOT between 2002 and 2019, 48 (91%) patients had neuroinvasive disease (mostly encephalitis or meningoencephalitis) [135]. Another review of 69 cases of WNV in SOT also found neuroinvasive disease in 61 (88%) patients [133]. Although it should be noted that most milder and asymptomatic cases likely do not get reported.

In a review of 52 published cases of WNV in SOT, the median time to infection from transplant was 14 months, and the mean age of patients was 50 years [135]. The mean time to diagnosis from symptoms onset was 5.2 days. Presenting symptoms in SOT can be nonspecific even in patients who eventually develop neurological illness [136–139]. In a study of 24 neuroinvasive infections in SOT, 88% and 71% of patients presented with gastrointestinal symptoms and fever, respectively [138]. Only 4 (17%) had cognitive impairment and 2 (8%) had acute flaccid paralysis at presentation. The median time to clinical worsening after admission was 4 days (range 1–11 days). Abnormal movement including myoclonus

and parkinsonian features can occur in some patients [135,139]. In a retrospective study of neuroinvasive disease, immunocompromised patients were less likely to have headache and myalgia and more likely to have myoclonus and encephalopathy compared to non-immunosuppressed patients [140].

There is no clear association of WNV with acute cellular rejection, however, rejection may result from a reduction in immunosuppressants following infection (118, 122, 125). There can be slight decrease in renal function in KT recipients with WNV infection, but graft loss attributable to WNV is uncommon [141]. In one study of SOT, allograft loss occurred in 2 (4%) of 52 patients [135]. In the same study, the overall mortality was 37%. In another review of SOT with WNV, 18 (31%) out of 59 patients with known outcome died [133]. All patients who died had neuroinvasive disease where 18 (33%) out of 55 patients with known outcome died. This mortality rate is higher than that of general population where the overall mortality rate associated with neuroinvasive disease is 9% [130]. Other studies have also shown higher mortality in neuroinvasive disease although mortality seems variable in smaller studies [134,138,139,141,142]. In general, higher mortality is seen in immunocompromised patients [143]. A recent study of neuroinvasive disease also showed longer duration of hospitalization and higher rates of ICU admission, mechanical ventilation, and 90-day all-cause mortality in immunocompromised patients compared to non- immunocompromised patients [140]. As in the general population, mortality is higher among patients with acute flaccid paralysis and encephalitis than meningitis alone [138]. Neurologic deficit, including permanent damage, can be significant among survivors [135,138,139,141].

In neuroinvasive disease, brain MRI can show punctate subacute infarcts and T2 flair hyperintensity signals in the brain stem, thalamus, cerebellum, and mesial temporal lobes [137–140]. CSF can be abnormal with mild pleocytosis and elevated protein [137]. CSF white blood cell count tends to be higher in immunocompromised patients [140]. The CSF pleocytosis is neutrophilic predominant early on before transitioning to lymphocytic predominant [138]. The CSF glucose in generally within normal range.

2.8.3. Diagnosis

WNV nucleic acid amplification test (NAT) or PCR can be performed in blood, CSF, and tissue. Next generation sequencing can also be performed in CSF. In general, WNV viral load is higher, and the viral RNA persists longer in whole blood compared to plasma [144]. The presence of nucleic acid in body fluids can be transient and WNV IgM (preferably with confirmatory PRNT) in blood and CSF is relied on for diagnosis at a later stage. In a study of 24 SOT with neuroinvasive disease, serum and CSF WNV IgM was positive in 63% and 48%, respectively, in whom it was tested [138]. In total, 8 (89%) out of 9 patients had positive serum NAT, while 8 (38%) out of 21 patients had a positive serum PCR. CSF PCR was positive in 44% of tested patients. In another review of SOT with WNV infection, serum RNA was positive in 10 (83%) out of 12 patients and serum IgM was positive in 15 (83%) out of 18 patients [137]. In patients with neuroinvasive disease 6 (75%) out of 8 patients and 10 (71%) out of 14 patients tested positive for CSF WNV RNA and IgM. In one study of kidney and or pancreas recipients, 17 out of 19 patients mounted a serologic response within 2–4 weeks [136]. In patients with neuroinvasive disease, positive CSF PCR tends to be more frequent in immunocompromised patients [140]. It is important to note that serological and RNA positivity depends on the timing of the test in relation to symptom onset, and negative tests do not rule out diagnosis. Other diagnostic tests include metagenomics in CSF, immunohistochemistry, and PCR on tissue [137,145–148]. Viral culture is rarely performed.

2.8.4. DDI

In a retrospective study of DDI in 207 KT recipients from 139 donors between 1948 and 2017, WNV accounted for 13 (6.3%) infections, of which 5 (38%) died [149]. In a review of donor-derived diseases by the Disease Transmission Advisory Committee between 2008

and 2017, WNV accounted for 5 (2%) out of 250 proven and probable pathogens transmitted from 244 donors [150]. Although the estimated donor derived proven and probable WNV occurred in only 0.17 per 100,000 SOT recipients between 2012 and 2017, sporadic clusters of donor-derived WNV continue to occur. Abbas et al., in their review, noted that DDI accounted for a quarter of the 53 published cases of WNV [135]. To date, there are no reported cases of transmission of WNV through living donors [134].

The first cases of DDI were reported in 2002 in four SOT recipients from a common organ donor who had received blood transfusion from an infected donor prior to organ procurement [148]. DDI occurs in the immediate post-transplant period with an incubation period of (median) 13 days, and initial symptoms can be non-specific [137]. Soto et al. recently reported 2 cases of DDI and reviewed 21 previously published cases with adequate information [134]. Two of the eight organ donors became infected via blood transfusion prior to organ procurement, and the rest were assumed to be infected via mosquito bites. Overall, 23 (85%) out of 27 SOT recipients from 10 infected donors acquired infection, and 16 (70%) developed neuroinvasive disease. Six patients died with an overall mortality of 26% (38% among patients with neuroinvasive disease), like that of non-DDI.

2.8.5. Pre-Transplant Screening

All potential donors should be screened for signs and symptoms of and risk factors for WNV exposure (e.g., travel to an endemic area). In the United States, the Organ procurement and Transplantation Network ad hoc Disease Transmission Advisory Committee recommends pre-transplant screening of both living and deceased donors with plasma WNV NAT during periods of heightened WNV activity [151]. Year-round WNV screening can, however, increase false positive rates, and it is discouraged to avoid inappropriate discarding of organs. Either seasonal testing (July through October in the continental United States) or triggered testing during increased WNV activity in the community (where the donor has lived or traveled to) can be performed. In deceased donors, however, the result may not be available at the time of transplantation, and not all organ procurement centers routinely screen for WNV infection in deceased donors. In one survey only 39% of 46 centers screened for WNV infection in potential deceased donors [152]. In living donors, plasma WNV NAT should be performed within 7–14 days prior to transplantation [151]. If NAT is positive, then the transplantation should be deferred for 28 days after which plasma NAT should be repeated and WNV IgM checked. If NAT remains positive, organ donation should be deferred. If both NAT and IgM are negative (likely false positive initial NAT test) or NAT is negative and IgM is positive (recovery from WNV infection), organ donation can be considered.

In the review by Soto et al., 9 out of 10 donors who transmitted WNV did not undergo pre-transplant testing. The archived serum on retrospective testing was positive for viral RNA in only six donors. In four donors, the transmission occurred despite negative viral RNA in the sera. Hence, despite pre-transplant screening for viral RNA, disease transmission can occur. However, screening will at least eliminate the infected donors who test positive. Serum/plasma RNA can be falsely negative in potential deceased donors due to dilutional effect from resuscitation measures or low-level RNAemia below the threshold for detection. Moreover, WNV can be detected in various organs or tissues by PCR, immunohistochemistry, and viral culture despite clearance from the blood [129,145,146,153]. WNV RNA has also been detected in urine for years after recovery from infection suggesting that kidneys can be a reservoir for viral reactivation [154]. In one instance of DDI, WNV was isolated in culture from lymph node/spleen tissue despite negative RNAemia [137,146]. A positive serum IgM/PRNT is suggestive of viral clearance from the blood, but as reviewed by Soto et al., viral transmission occurred from three donors with negative serum RNA who had mounted an IgM response. Thus, potentially replicative virus harboring in organs cannot be detected in donors with negative plasma or serum NAT and positive serum IgM, and this creates a challenge in organ transplantation [155]. Hence, careful assessment of donors should be performed to minimize the risk of transmission. It should be noted that

potential donors with WNV infection may not have encephalopathy, and confounding diagnoses in donors with encephalopathy might lead to classifying them as low risk for disease transmission [149,156].

2.8.6. Prevention

Mosquito control: See Japanese encephalitis section on cullex mosquito prevention (Section 2.2.3).

No vaccines are currently available for WNV prevention.

2.8.7. Treatment

Treatment is supportive. IVIG, hyperimmune globulin, steroids, and interferons have been used in SOT with WNV infection, but data supporting their efficacy are lacking [140,157,158]. Immunosuppressants are reduced in >90% cases in published literature but its effect on survival is not clear [135].

2.9. Other Arboviruses

There are scattered case reports of less common arbovirus infection in SOT.

A cluster of donor-derived Eastern equine encephalitis (EEQ) viral infection has been reported in three SOT recipients (heart, liver and lung) [159]. All three organ recipients developed encephalitis within a week of transplant. The clinical course in the heart transplant recipient was complicated by acute cellular rejection or myocarditis. All recipients were treated with IVIG +/− steroids. Only the lung transplant recipient survived with residual neurologic deficit. The donor likely acquired the infection via mosquito bite and pre-transplant stored donor serum tested positive for EEQ virus RNA.

A few cases of encephalitis caused by St Louis encephalitis (SLE) virus (acquired via blood transfusion in the post-transplant period or mosquito bite) with high morbidity and mortality has been reported in SOT [160,161].

Similarly, Jamestown Canyon virus has been reported to cause encephalitis in a heart and a liver transplant recipient 4 and 3 years after transplant, respectively [162,163]. The infection was presumably acquired via a mosquito bite. Both patients survived.

The Cache Valley virus has been reported to cause meningoencephalitis in a KT recipient in the immediate post-transplant period [164]. The infection was thought to be acquired via blood transfusion on the day of transplant from an infected blood donor.

A fatal case of Crimean–Congo hemorrhagic virus, confirmed by a positive viral RNA in the blood, has been reported in a liver transplant health care worker who acquired it while performing surgery on a presumably infected patient [165].

Heartland virus infection has been reported in a heart transplant patient 7 years after transplant, likely to have occurred via a tick bite [166]. The patient presented with fever, leukopenia with lymphopenia, thrombocytopenia, transaminitis, myositis, and encephalopathy, and they eventually recovered. The diagnosis was confirmed by a positive blood and serum PCR.

Rift valley fever has bene described in a KT recipient returning traveler from Mali [167]. The mode of acquisition remained undetermined, but the patient endorsed mosquito bites, ingestion of raw milk, and contact with animals—all known risk factors for rift valley fever virus transmission. The patient presented with non-specific febrile syndrome that progressed to encephalitis. The patient made a full recovery. The diagnosis was supported by positive serum and CSF serology and confirmed by positive urine and semen PCR that remained positive for several weeks.

Usutu virus is an uncommon arbovirus that has been reported to cause encephalopathy in a liver recipient [168]. The liver recipient had undiagnosed Usutu infection at the time of transplant. The diagnosis was made by positive plasma PCR and gene sequencing. The virus was also isolated in cell culture (which on sequencing was identical to the post-transplant sequencing) from stored pre-transplant plasma and on sequencing.

Although no infections in transplant recipients have been reported to date, the recent explosion of the Oropouche virus in regions of South America, particularly Brazil, raise concern going forward [169].

2.9.1. Diagnosis

As with other arboviruses, diagnosis is made by serum, blood, CSF, and tissue PCR. Urine and semen RNA PCR can also be positive as was seen with rift valley fever viral infection in a KT recipient [167]. CSF metagenomics have also been used in diagnosis [164]. Viral culture is cumbersome but can be performed, especially, in early infection [164,168]. Gene sequencing can be used for confirmation [164,168]. Blood/serum and CSF IgM is supportive of the diagnosis and should be confirmed with neutralizing antibodies like PRNT. Tissue immunohistochemistry has also been used to aid diagnosis [159].

2.9.2. Prevention

Mosquito control, avoidance of mosquito bites, and ingestion of unpasteurized dairy products are recommended. Ribavirin has been used for prophylaxis in those exposed to Crimean–Congo hemorrhagic fever virus [170]. An inactivated vaccine against the Crimean–Congo Hemorrhagic virus is available in Bulgaria, but its effectiveness is uncertain [170].

2.9.3. Donor Screening

Routine donor and recipient screening is not recommended (see Section 2.2).

2.9.4. Treatment

Treatment is supportive. Although IVIG, ribavirin, favipiravir, hyperimmune globulin, and interferons have been used with antecedental reports of success, there are no strong data to support their routine use [159–165,170]. It may be prudent to reduce immunosuppressants during active infection, but the role of this strategy in outcome is not clear [166].

3. Conclusions

The clinical course of arbovirus in SOT is generally more severe and carries a higher mortality compared to that of the general population. However, the data on arboviruses are limited, and asymptomatic and milder illnesses likely go undetected. Diagnostic tests, like PCR and NAT, are transiently positive in most cases, and IgM tests suffer from poor specificity. Confirmatory neutralizing antibodies like PRNTs are not widely available. Even PRNT may not be able to distinguish the causative viral agent from another recently exposed similar arbovirus. Ideally paired acute and convalescent specimens are required for serological testing to confirm acute infection by demonstrating seroconversion and a fourfold rise in titer, but this may not be pragmatic and does not provide diagnosis in real time. Moreover, most of these assays may not be available in resource-limited settings. SOT recipients also face a unique situation where they can acquire infection from donor allografts as well as blood donors in addition to de novo infection caused by exposure to the vector during community outbreaks of arboviruses. This poses a challenge to transplant centers in selecting and accepting appropriate donor organs. While implementation of screening strategies in organ donors to prevent transmission of virus to transplant recipients is necessary, overzealous screening and imperfect diagnostic tests can lead to false positive tests and discarding of potentially lifesaving organs.

4. Future Direction

More data on arbovirus infection in this population and better diagnostic tests and effective therapeutics are needed.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/v16111778/s1, Supplementary Material S1: Methods used in selecting articles for the review.

Author Contributions: K.G. and R.D. reviewed literature and drafted the manuscript. S.D. critically reviewed the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: S.D.-none; K.G.-consultant to Karius (but not relevant to this manuscript). R.D. is an employee of labcorp, but this study is performed independently.

Abbreviation

AKI	acute kidney injury
CHKV	Chikungunya virus
CSF	cerebrospinal fluid
DENV	dengue virus
DDI	donor derived infection
EEQ	eastern equine encephalitis
IVIG	intravenous immunoglobilin
JE	Japanese encephalitis
JEV	Japanese encephalitis virus
KT	kidney transplant
NAT	nucleic acid amplification test
PRNT	plaque reduction neutralization test
SLE	St Louis encephalitis
SOT	solid organ transplant
TBE	tick borne encephalitis
TBEV	tick borne encephalitis virus
WHO	World Health Organization
WNV	west nile virus
YF	yellow fever
YFV	yellow fever virus
ZKV	zika virus

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viruses

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Correction

Correction: Gajurel et al. Arbovirus in Solid Organ Transplants: A Narrative Review of the Literature. *Viruses* 2024, *16*, 1778

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Error in Table

In the original publication, there was a mistake in Table 1 as published [1]. Mayaro virus was mistakenly listed under *Flaviviridae* family. It should have been listed under '*Togaviridae* family'. Also, under *Bunyaviridae* family (Bunyavirales), "Rift Vallet fever" virus should be corrected to "Rift Valley fever virus". The correct Table 1 is as follows.

Table 1. Medically significant arboviruses.

***Togaviridae* family:**
Eastern equine encephalitis virus
Western equine encephalitis virus
Venezuelan equine encephalitis virus
Chikungunya virus
Sindbis virus
Ross river virus
Barmah Forest virus
Mayaro virus
***Flaviviridae* family:**
Dengue virus
Japanese encephalitis virus
Murray valley fever virus
St Louis encephalitis virus
West Nile virus
Powassan virus
Tick Borne encephalitis virus
Louping ill virus
Omsk hemorrhagic fever virus
Alkhurma Hemorrhagic Fever Virus
Kyasanur Forest Disease Virus

Table 1. *Cont.*

Zika virus
Yellow fever virus
Usutu virus
Rocio encephalitis virus
***Bunyaviridae* family (Bunyavirales)**
California encephalitis virus
-La Crosse encephalitis virus
-Jamestown Canyon
Rift Valley fever virus
Crimean Congo hemorraghic fever virus
Severe fever with thrombocytopenia syndrome
Heart land virus
Oropouche Virus
Toscana Virus
Cache Valley fever
Ngari Virus
***Reoviridae* family**
Coltivirus
-Colorado tick fever virus
-Seadornavirus (Banna virus)
***Orthomyxoviridae* family**
Thogotovirus genus including thogoto, bourbon and dhori viruses

Reference

1. Gajurel, K.; Dhakal, R.; Deresinski, S. Arbovirus in Solid Organ Transplants: A Narrative Review of the Literature. *Viruses* **2024**, *16*, 1778. [CrossRef] [PubMed]

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4052 Basel
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Viruses Editorial Office
E-mail: viruses@mdpi.com
www.mdpi.com/journal/viruses

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ISBN 978-3-7258-4530-9