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CMV management of patients with leukopenia after CMV high-risk kidney transplantation

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ABSTRACT

Background: For CMV high-risk constellations, guidelines recommend 3–6 months of prophylaxis with valganciclovir (VGCV). Management in preventing CMV primary infection in patients developing VGCV-associated leukopenia remains challenging.
Methods: We retrospectively analyzed the development of leukopenia during VGCV prophylaxis in 57 seronegative kidney recipients of a CMV-seropositive donor between 2008 and 2021. We analyzed CMV risk and development of CMV-specific T cells in the first post-transplant year depending on leukopenia during VGCV prophylaxis and management with CMV-IVIg.
Results: Leukopenia developed in 19/57 patients, with a significant difference in leukocyte counts occurring after 10 weeks of VGCV prophylaxis compared to patients without leukopenia ($p = 0.0003$). VGCV discontinuation led to leukocyte reconstitution, which tended to be faster in patients receiving additional prophylaxis with CMV-IVIg after VGCV discontinuation ($n = 11$, $p = 0.083$). In the first post-transplant year, patients with leukopenia had no higher risk for severe CMV events. Interestingly, patients receiving CMV-IVIg prophylaxis showed a significantly lower peak CMV-load during primary infection ($p = 0.040$), with no difference in CMV-specific T-cell levels compared to patients without leukopenia or patients with additional CMV-IVIg prophylaxis ($p = 0.972$). Patients developing adequate CMV-specific T-cell responses less frequently underwent CMV reactivation 50 days following primary infection.
Conclusion: Leukopenia developed late during VGCV prophylaxis and did not result in an increased risk for CMV primary infections or severe disease. Leukopenic patients receiving CMV-IVIg tended to have a faster leukocyte reconstitution and had lower peak DNAemia, which did not adversely affect CMV-specific T-cell induction. CMV-IVIg may therefore be considered as an alternative prophylactic strategy in patients with VGCV-associated leukopenia.

1. Introduction

Cytomegalovirus (CMV) management after kidney transplantation has improved considerably in recent decades based on the availability of drugs such as ganciclovir and valganciclovir (VGCV). However, there are still challenges in the management of seronegative recipients receiving an organ from a seropositive donor, who are among the patients with the highest risk, and their proportion is increasing due to decreasing seroprevalence in the general population [1]. For CMV high-risk constellations, the current guidelines recommend three to six months of CMV prophylaxis with VGCV to prevent CMV primary infections during the early post-transplant period with highest immunosuppression [2,3]. However, side effects of VGCV may occur that limit compliance with the recommended duration of prophylaxis. Leukopenia is among the most common side effects that often leads to early discontinuation of VGCV prophylaxis [3–5], which necessitates

Abbreviations: CMV, cytomegalovirus; CMV-IVIg, CMV i.v. immunoglobulins; EOD, end organ disease; IQR, interquartile range; VGCV, Valganciclovir.

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alternative strategies for prevention of primary infection. If primary infection occurs, induction of CMV-specific T cells is essential for long-term control of viral replication [6]. Observational studies have shown that leukopenia primarily occurs after approximately 10 weeks of prophylaxis with VGCV [7,8]. Knowledge on the CMV risk after discontinuation of prophylaxis due to leukopenia and on the value of alternative management strategies in high-risk patients is limited. Although not routinely recommended by guidelines [2], CMV i.v. immunoglobulins (CMV-IVIg) have been used for prophylaxis in patients with VGCV-associated myelosuppression [2]. The effect of CMV-IVIg on viral load during primary infection and induction of CMV-specific immunity is unknown. In this monocentric retrospective study, we therefore analyzed the incidence of leukopenia during VGCV prophylaxis. Both patients with and without leukopenia as well as leukopenic patients managed with and without CMV-IVIg after discontinuation of VGCV, were compared regarding incidence of CMV infection, viral load and the induction of CMV-specific immunity.

2. Methods

2.1. Subjects and study design

We conducted a retrospective monocentric study among high-risk kidney transplant recipients transplanted at Saarland University medical center between 2008 and 2021. The CMV-IgG status of the recipient and the donor were determined prior to transplantation. As per center standard, patients receive three months of VGCV prophylaxis. In case of discontinuation of VGCV due to adverse events, patients may receive alternative prophylaxis with CMV-IVIg. All patients underwent CMV-DNA testing before stopping prophylaxis, or in case of any febrile episode or other signs or symptoms compatible with CMV primary infection during prophylaxis. CMV-DNAaemia and CMV-specific CD4 T cells were determined biweekly during the first 6 weeks after stopping prophylaxis or in case of signs or symptoms compatible with CMV primary infection. CMV-IgG seronegative transplant recipients ($\geq$18 years old) were included if they had received CMV prophylaxis with VGCV after transplantation of a kidney from a CMV-IgG seropositive donor and had graft survival in the first six months after transplantation. Information on therapeutic immunosuppression, and clinical data related to transplantation outcome during the first year (CMV primary infection, CMV end organ disease (EOD), rejection) were collected retrospectively from electronic medical records. Furthermore, routine laboratory data such as leukocyte counts during CMV prophylaxis as well as CMV-DNA and CMV-specific CD4 T-cell levels were evaluated. The study was approved by the ethics committee of the Ärztekammer des Saarlandes with patient consent considered dispensable due to the retrospective nature of the study (reference no. 249/20).

The incidence of CMV primary infections and of CMV EOD in the first year after transplantation as well as the induction of CMV-specific T cells was investigated in patients with and without leukopenia. CMV events in patients after regular VGCV prophylaxis were compared with patients on alternative management strategies.

2.2. Quantification of CMV-DNA and CMV-specific CD4 T cells

CMV-DNA was performed as part of routine clinical diagnostics using the Cobas Amplicor assay (Roche Diagnostics, Mannheim, Germany) adapted to international units (IU) per milliliter blood as per international WHO standard [9]. CMV-specific CD4 T cells were measured after a 6 h stimulation of heparinized whole blood with a CMV-lysate using an in-house assay as part of routine clinical diagnostics. The assay is based on identification of CMV-specific CD4 T cells using flow cytometry by co-expression of CD69 and IFNγ after stimulation with a commercially available lysate of CMV infected fibroblasts (Virion, Würzburg, Germany) as described previously [10]. CMV-specific T-cell levels $\geq$0.05 % after subtraction of reactive cells after stimulation with a lysate of non-infected cells (control antigen, Virion, Würzburg, Germany) were scored as positive. This threshold yielded concordant results with CMV IgG serology in 99.5 % of tests as previously described [11].

2.3. Definition of variables

The following definitions of variables were used: Leukopenia was defined as leukocyte counts $<3.9 \times 10^6$ per milliliter whole blood in at least two consecutive measurements on two different days. The start of leukopenia was defined as the first measurement $<3.9 \times 10^6$/ml leukocytes and the end of leukopenia was defined as first time leukocytes were $\geq 3.9 \times 10^6$/ml confirmed by at least one second measurement on two different days. CMV primary infection was defined as any detectable DNAemia after transplantation, and the start of CMV primary infection was defined as the time point, when the first time CMV-DNA was detected. The end of primary CMV infection was defined as the end of antiviral therapy, which ended after two consecutive negative detections of CMV-DNA at least 7 days apart [2]. EOD (histologically proven or possible EOD) was defined according to current guidelines [12] as clinical symptoms combined with histology or CMV-DNA detection from specimens of the specific organ system that included tissue biopsies or body fluids. Information on CMV resistance was only available if resistance testing was verified and documented. Clinically significant CMV infections were defined as CMV primary infection regardless of the quantity of CMV-DNA, CMV-DNA $\geq$1000 UI/ml blood after end of primary infection or EOD. CMV-DNA detection after end of CMV primary infection with DNAemia <1000 UI/ml blood in the absence of clinical symptoms was considered as clinically non-significant infection.

2.4. Statistical analysis

All statistical analyses were performed using GraphPad Prism 10.0.2.232 software (GraphPad, San Diego. CA, USA) using two-tailed tests. Categorical analyses on sex and adverse events were performed using Fisher's exact-test. Data with normal distribution were analyzed using unpaired t-test. To compare unpaired nonparametric data between groups, Mann-Whitney and Kruskal–Wallis test followed by Dunn's multiple comparisons test were performed. Wilcoxon matched pairs test was used to compare paired data between two groups. For endpoint analyses, survival analyses were performed using Log-rank-test. A p-value less than 0.05 was considered statistically significant.

3. Results

3.1. Study population

Fifty-seven seronegative patients receiving CMV prophylaxis with VGCV after kidney transplantation from a CMV-seropositive donor were consecutively included, of which 19 developed leukopenia (fig. S1A). Patient characteristics and underlying diseases are shown in Table 1. Patients who developed leukopenia did not differ in age and sex (51.2 $\pm$ 17.2 years; 42.1 % females) compared to patients without leukopenia (53.4 $\pm$ 14.4 years; 31.6 % females). Most patients had received a kidney from a deceased donor (80.7 %) and underwent hemodialysis before transplantation (76.9 %). Only 8.8 % had AB0 incompatible kidney transplantation and 13.0 % were pre-immunized. Most patients (92.1 %) received induction therapy with IL-2 receptor antibodies, while only few patients had induction with ATG ($n = 2$) or rituximab ($n = 1$; Table 1). The standard immunosuppressive drug regimen after transplantation included 2000 mg MMF per day, tacrolimus with a target level of 5–10 ng/ml in the first three months post-transplantation with subsequent reduction to 4–7 ng/ml, and methylprednisolone that was tapered from 625 to 4 mg during the first 71 days.

Table 1
Demographic and clinical characteristics of the study population.

		no leukopenia	leukopenia	p-value
		$n = 38$	n = 19	
Years of age, mean (SD)		53.4 (14.4)	51.2 (17.2)	0.619†
Sex¶, n (%)				
	female	12 (31.6 %)	8 (42.1 %)	0.558‡
	male	26 (68.4 %)	11 (57.9 %)	
Basic nephrological disease, n (%)				
	congenital	6 (15.8 %)	6 (31.6 %)	
	acquired disease	19 (50.0 %)	9 (47.4 %)	
	idiopathic	5	3	
	autoimmune	8	3	
	secondary	6	3	
	other	13 (34.2 %)	4 (21.1 %)	
Type of dialysis before kidney transplantation, n (%)				
peritoneal dialysis		6 (15.8 %)	6 (31.6 %)	0.300‡
hemodialysis		28 (73.7 %)	12 (63.2 %)	
unknown		4 (10.5 %)	1 (5.3 %)	
Type of donation, n (%)				
living		6 (15.8 %)	5 (26.3 %)	0.478‡
deceased		32 (84.2 %)	14 (73.7 %)	
Pre-immunization, n (%)				
	no	29 (76.3 %)	18 (94.7 %)	0.400‡
	yes	6 (15.8 %)	1 (5.3 %)	
	unknown	3 (7.9 %)	0	
AB0 incompatibility, n (%)				
	yes	4 (10.5 %)	1 (5.3 %)	0.656‡
	no	34 (89.5 %)	18 (94.7 %)	
Induction therapy, n (%)				
	basiliximab/ daclizumab	35 (92.1 %)	19 (100 %)	
	ATG	2 (5.3 %)		
	rituximab	1 (2.6 %)		
duration of VGCV prophylaxis (median weeks, IQR)		12.6 (1.7)	12.4 (3.3)	>0.999§

¶ assigned at birth.
† unpaired t-test.
‡ Fisher's exact test.
§ Mann-Whitney test.

3.2. Duration of VGCV prophylaxis and development of leukopenia under prophylaxis

All patients were targeted to receive at least 3 months of VGCV prophylaxis with doses adjusted to renal function from 450 mg every second day to 900 mg per day. The median duration of prophylaxis was 12.6 (interquartile range (IQR) 1.9) weeks with individual modification depending on the clinical course. A total of 19 patients developed leukopenia during prophylaxis, and discontinued prophylaxis. As shown in fig. S1B, leukocyte counts decreased to a similar extent in all patients until week 10. A significant difference in leukocyte counts between patients with and without leukopenia only became apparent by week 10–12. The median duration of VGCV prophylaxis in the group of 19 patients with leukopenia until discontinuation of VCGV was 12.4 (IQR 3.3) weeks with only two patients who had very early discontinuation at week 2.4 and week 7.6, respectively. As leukopenia generally occurred rather late, duration of prophylaxis in patients with leukopenia did not significantly differ from patients without leukopenia (median 12.6 (IQR 1.7) weeks, $p > 0.999$, Table 1). Among the 38 patients without leukopenia, 35 had their anticipated time of prophylaxis, while only three had VGCV prophylaxis terminated earlier due to CMV primary infection at week 4.3, for VGCV-associated hepatotoxicity at week 3.7 and for unclear reasons at week 7.9. The dosage of antiproliferative immunosuppressive drugs during prophylaxis did not differ in patients with and without leukopenia (table S1).

Among the 19 patients who discontinued prophylaxis due to VGCV-associated leukopenia, 11 patients received between 2 and 4 weight-adapted doses of CMV-IVIg (100 IU/kg body weight) at a median interval of 14 (IQR 0.75) days) as supportive extension of prophylaxis (fig. S1A). Among those, two were switched back to VGCV prophylaxis after normalized leukocyte counts were achieved. The other 8 patients with leukopenia did not receive any additional prophylaxis. Among the 38 patients without leukopenia, two were also switched to CMV-IVIg prophylaxis. One was the patient with VGCV-associated hepatotoxicity (4 doses at an interval of 14 days), and one patient had a single CMV-IVIg infusion in the context of BKPyV virus infection, and less frequent visits in the ambulatory setting in times of lockdown during the SARS-CoV-2 pandemic.

3.3. Recovery of leukopenia after discontinuation of VGCV prophylaxis

Recovery of leukopenia occurred at a median of 1.86 (IQR 2.43) weeks after stopping VGCV prophylaxis. Patients with additional CMV-IVIg prophylaxis tended to reach normal leukocyte counts earlier than patients without further prophylaxis, although this difference did not achieve statistical significance ($p = 0.083$; fig. S2A). Apart from CMV-IVIg prophylaxis, the daily dose of MMF was also modified after discontinuation of VGCV prophylaxis based on the individual clinical course to avoid further myelotoxicity. This clinical intervention resulted in a comparable course of leukocyte reconstitution in all patients (fig. S2B).

3.4. CMV primary infection in the first year after transplantation

Overall, 32/57 patients had primary CMV infection in the first year after transplantation. Clinical data of patients without and with leukopenia, the latter stratified based on additional CMV-IVIg prophylaxis are shown in Table 2. There was no difference in the percentage of primary infections in patients with (9/19 (47.4 %)) and without leukopenia (23/38 (60.5 %); $p = 0.404$). Primary infection occurred at a median of 145 (IQR 138) days after transplantation. Four primary infections, all in patients without leukopenia, occurred while still on VCGV prophylaxis of which three occurred close to regularly stopping prophylaxis. All other CMV primary infections occurred after stopping VGCV prophylaxis with no difference in patients with and without leukopenia ($p = 0.224$, Fig. 1A). Primary infection in patients with leukopenia occurred after a median of 160 days (IQR 180) with no difference in patients with or without additional CMV-IVIg prophylaxis ($p = 0.413$, Table 2). Most patients with CMV primary infection had mild courses, and primary induction of CMV-specific CD4 T cells only became detectable concomitant with or after DNAemia (Fig. 1B). Treatment regimens for primary infection included GCV or VGCV with or without combination with CMV-IVIg or foscarnet (table S2).

3.5. CMV-DNA and development of CMV-specific CD4 T cells after CMV prophylaxis

All patients underwent regular CMV-DNA testing before stopping prophylaxis, or in case of any febrile episode or other signs or symptoms compatible with CMV primary infection during prophylaxis. Analysis of CMV-specific CD4 T cells was performed at least once. CMV-DNA was

Table 2
CMV events and outcome of the study population.

	no leukopenia	leukopenia		p-value
	n = 38	CMV-IVIg prophylaxis n = 11	no further prophylaxis *n* = 8	
Primary infection in first year after transplantation (n, %)	23 (60.5 %)	5 (45.5 %)	4 (50.0 %)	0.628‡
first detection of CMV-DNA after transplantation (median in weeks, IQR)	20.0 (11.0)	22.9 (21.6)	34.0 (30.0)	0.444†
Days between end of CMV prophylaxis§ and primary infection (median, IQR)	48 (61)	0 (103.5)	113 (117.5)	0.219†
peak DNAemia (IU/ml) during primary infection, (median, IQR)	12,000 (58100)	4500 (10050)	39,500 (213500)	0.040†
EOD during primary infection n (%)	2¶ (8.7 %)	0	0	
CMV resistance during primary infection, n (%)	1$ (4.4 %)	0	0	
Duration of primary viremia, (median in weeks, IQR)	10.9 (23.9)	6.0 (20.7)	8.8 (26.4)	0.7†
Days between first positive CMV-DNA until first CMV-specific T-cell detection (median, IQR)	12 (79)	2 (90)	11 (37.5)	0.8†
Reactivation 50 days after primary infection (n, %)	7 (30.4 %)	2 (40.0 %)	1 (25.0 %)	0.879‡
outcome 3 years after transplantation	*n* = 35#	*n* = 11	n = 8	
graft survival	33 (94.3 %)	10 (90.9 %)	6 (75.0 %)	
graft failure	2 (5.7 %)	1 (9.1 %)	2 (25.0 %)	
recipient survival	35 (100.0 %)	11 (100.0 %)	7 (87.5 %)	
recipient mortality	0 (0 %)	0 (0 %)	1† (12.5 %)	

† Kruskal-Wallis test.
‡ X^2-test.
§ refers to any type of CMV prophylaxis with or without subsequent CMV-IVIg.
¶ 1 proven CMV colitis (by positive CMV-DNA in stool and immunohistochemisty), 1 possible CMV colitis (by positive CMV-DNA in stool).
$ Mutation in the UL54 gene (Q578H) conferring reduced sensitivity to ganciclovir, cidofovir and foscarnet.
# 3 patients were lost for follow-up.

consistently negative, except for 4/57 patients (7 %) who had CMV-DNA detected during VGCV prophylaxis, which resulted in switching to treatment dosage. CMV-specific CD4 T cells were absent in all patients prior to transplantation until the end of VGCV prophylaxis ($n = 57$). We next analyzed CMV-DNA load and induction of CMV-specific CD4 T cells in the subgroup of 32 patients with primary infection, and whether this differed in patients with and without history of leukopenia, the latter stratified based on additional administration of CMV-IVIg. By the end of VGCV prophylaxis and/or after additional CMV-IVIg prophylaxis, most patients were still CMV-DNA negative (Fig. 2A), and only one patient had detectable CMV-specific CD4 T cells after the end of CMV-IVIg prophylaxis (Fig. 2B). Peak DNAemia during primary infection was significantly different between the three groups ($p = 0.040$). Patients without leukopenia reached median peak levels of CMV-DNAemia of 12,000 (IQR 58100) IU/ml. Interestingly, among patients with leukopenia, peak DNAemia levels were significantly lower in the subgroup who had received CMV-IVIg ($p = 0.041$, Fig. 2A, Table 2). Despite this difference in viral load, all three patient groups reached similar levels of CMV-specific CD4 T cells ($p = 0.972$, Fig. 2B). Although 11/28 (39.3 %) patients were still CMV-specific T-cell negative at the time of peak DNAemia, most patients developed CMV-specific immunity by the time when primary DNAemia was controlled by antiviral treatment (22/28, 78.6 %, Fig. 2A/B). In general, all patients had developed CMV-specific CD4 T cells after a median of 21.1 weeks (IQR 20.1). The median peak DNAemia level preceding the time when CMV-specific CD4 T cells first became detectable was 11,500 UI/ml (IQR: 40125). Again, patients with leukopenia who had received CMV-IVIg prophylaxis had significantly lower maximum CMV-DNA loads than patients with leukopenia who did not receive CMV-IVIg ($p = 0.027$, Fig. 2C), while the level of CMV-specific T cells did not differ between the groups (Fig. 2D). This indicates that a lower peak viral load does not adversely affect primary induction of CMV-specific CD4 T cells.

We finally assessed the importance of CMV-specific CD4 T cells in predicting relapses. Within 50 days after treatment of primary infection, 10/32 had a clinically significant reactivation or possible EOD. As shown in Fig. 1E, these patients had significantly lower levels of CMV-specific CD4 T cells at the end of treatment for primary infection as compared to patients where CMV-DNA remained negative or had non-significant CMV reactivation ($p = 0.025$), indicating that low levels of specific T cells are associated with higher risk for relapse after control of primary infection.

Long-term follow-up revealed a low 3-year mortality (1/57) and low 3-year graft failure rate (5/57). The patient who died was lost for follow-up, but died in an external hospital after 2.75 years by unclear cause. Graft losses in 3/5 patients were unrelated to CMV (two with BKPyV nephritis and one with prerenal failure). Graft failure was CMV-related in two patients of which one had a CMV-resistant strain (UL54 Q578H, after 9 months), and one had CMV colitis during reactivation 30 months after transplantation (Table 2).

4. Discussion

VGCV prophylaxis is most frequently used as management strategy to prevent CMV complications in seronegative recipients of a seropositive organ. Although VGCV prophylaxis is associated with a significant reduction in the incidence of CMV primary infections in periods of highest immunosuppression, development of leukopenia is among the most frequent side effects which necessitates discontinuation of VGCV and the use of alternative prevention strategies [5,7]. This was also confirmed in our retrospective study, where leukopenia occurred in one third of all patients receiving VGCV prophylaxis. A decrease in leukocyte counts only occurred after more than seven weeks. There was no evidence that patients with leukopenia had an increased risk of primary infection or severe CMV disease in the first year after transplantation. Patients who received CMV-IVIg as an alternative prophylaxis for VGCV-related leukopenia had lower maximum CMV-DNAemia levels preceding the first detection of CMV-specific T cells than leukopenic patients without further prophylaxis or patients without leukopenia. Nevertheless, this did not have any adverse effects on the induction of CMV-specific T cells.

In general, toxicities associated with VCGV prophylaxis requires

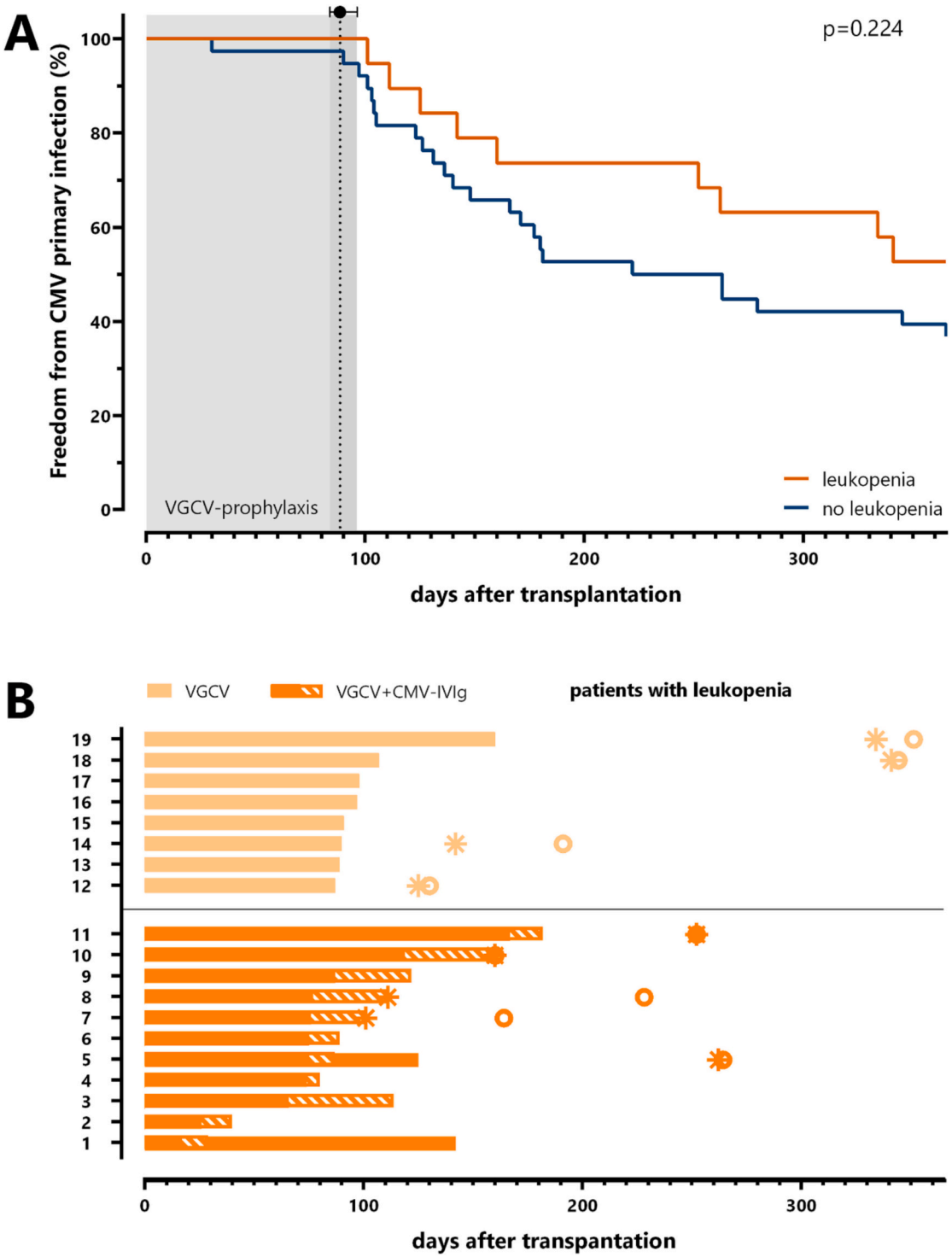

Fig. 1. CMV primary infection in the first year after transplantation. (**A**) Time to primary CMV infection is shown in patients without leukopenia on regular VGCV prophylaxis ($n = 36$) and in patients with leukopenia ($n = 19$). The median duration of VGCV prophylaxis of all patients is indicated by the shaded area. (**B**) Duration of CMV prophylaxis (VGCV±CMV-IVIg), start of primary CMV infection, and first detection of CMV-specific T cells in the first year after transplantation is shown in patients with leukopenia during VGCV prophylaxis. Patients are stratified according to whether or not they received additional prophylaxis with CMV-IVIg. Patients without further prophylaxis after discontinuation of VGCV (n = 8) are stratified from patients who received additional CMV-IVIg prophylaxis (n = 11). Bars refer to the duration of VGCV prophylaxis (with periods of CMV-IVIg prophylaxis shown as shaded areas). First detection of CMV-DNA is marked with a star and first detection of CMV-specific T cells is marked with a circle. Statistical analysis was performed by Log-rank (Mantel-Cox) test.

alternative management strategies to prevent CMV infections in high-risk patients. Given its known direct effects in mediating a variety of clinical symptoms and indirect effects in adversely affecting long-term graft survival, this also includes prevention of high-level replication [13]. In our center, patients with leukopenia were either subjected to a pre-emptive strategy and/or received additional prophylaxis with CMV-IVIg after stopping VGCV prophylaxis, which proved as viable option to prevent CMV disease. First, in line with other studies [7,8,14], leukopenia had developed rather late, when immunosuppression was already tapered in most patients. Second, a pre-emptive strategy has generally

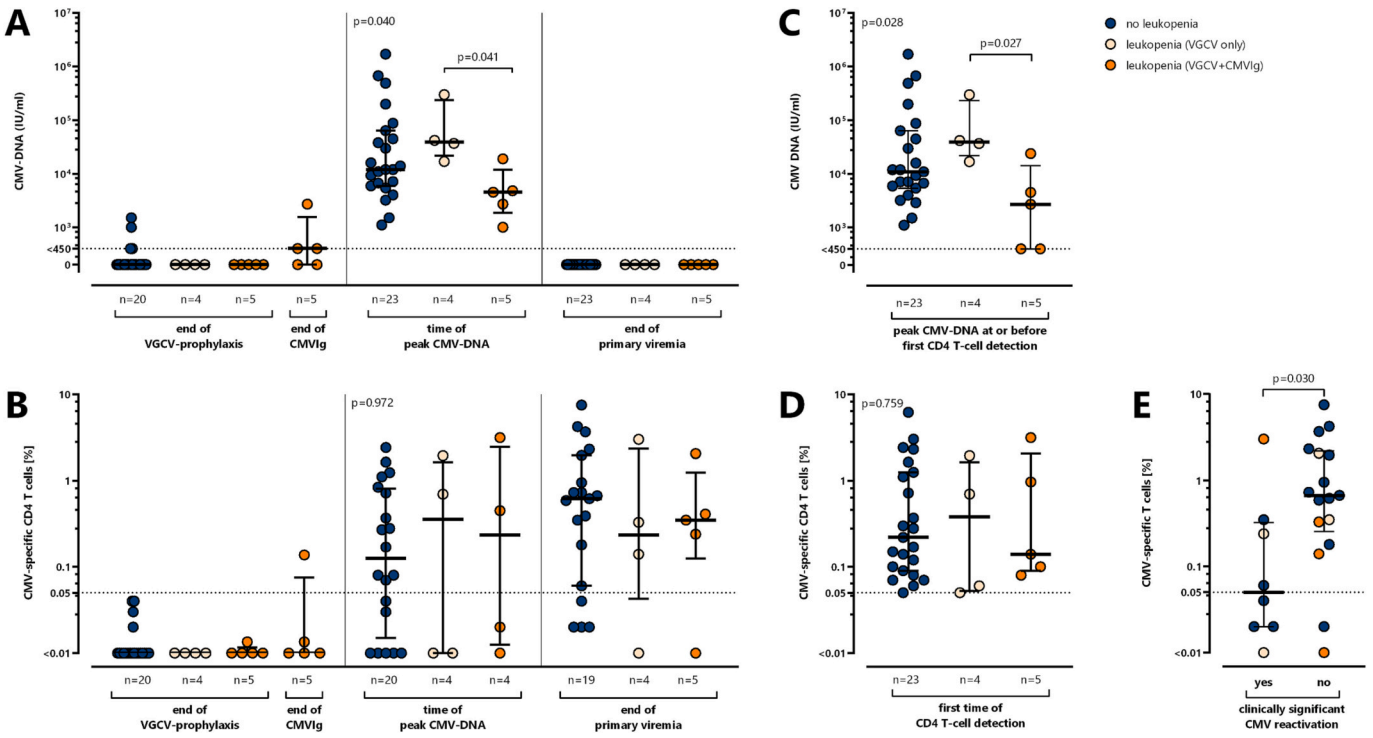

Fig. 2. CMV-DNAemia and CMV-specific T cells in patients with primary CMV infection in the first year after transplantation. CMV-DNAemia (**A**) and CMV-specific T cells (**B**) are shown at the end of CMV prophylaxis, at the time of maximal CMV-DNAemia, and at the end of primary CMV infection in patients with and without leukopenia, the former stratified by additional prophylaxis with CMV-IVIg. Moreover, CMV-DNA (**C**) and CMV-specific T cells (**D**) are shown by the time CMV-specific T cells became first detected. (**E**) CMV-specific T-cell levels at the end of primary CMV infection in patients who subsequently (within 50 days) developed a clinically relevant CMV infection (CMV-DNA load ≥1000 IU/ml blood or possible CMV end organ disease) compared to patients with non-relevant or no reactivations (non-detectable CMV-DNA or CMV-DNA <1000 IU/ml blood).

been recognized as not inferior to universal prophylaxis in liver transplant recipients [15], and in a recent meta-analysis among kidney transplant recipients [16], if regular monitoring or DNAemia and prompt initiation of VCGV therapy is ensured. More recently, prophylaxis with letermovir was shown to be noninferior to VGCV in preventing CMV disease [7]. Despite its approval, its higher cost may prevent its widespread use as an alternative to VGCV. However, the significantly lower rates of leukopenia or neutropenia [7] may help to advance its use in patients with VGCV-related toxicities.

Leukopenia resolved in all cases after discontinuation of VGCV prophylaxis regardless of adjustment of immunosuppression, suggesting that leukopenia in our cases was mainly VGCV-associated and that concomitant reduction in immunosuppression alone does not appear sufficient to resolve leukopenia. Interestingly, recipients with subsequent CMV-IVIg prophylaxis tended to regain normal leukocyte counts faster than those without further prophylaxis, but this observation would need to be confirmed in larger preferably randomized studies. Although there is currently no evidence for a general myeloproliferative effect of CMV-IVIgs [17], a potential contribution in resolution of leukopenia warrants further study.

One concern associated with leukopenia and the discontinuation of VGCV is a higher severity of CMV primary infection. In the whole cohort, primary CMV infection developed in more than 50 % of patients in the first year of transplantation but did not occur more frequently in patients with leukopenia. Infections were mild in almost all patients, and it was remarkable that no patient with a history of leukopenia had a symptomatic primary infection. Given the delay in developing leukopenia until week 10 in the majority of patients, this may result from the fact that the duration of prophylaxis was not significantly different in patients with and without leukopenia. Apart from preventing primary infection in the first place, early control of replication is important to prevent high-level DNAemia and emergence of resistant viruses during treatment. In this regard it is interesting to note that primary infections in patients who had received CMV-IVIg had significantly lower peak CMV-DNAemia levels than patients managed without CMV-IVIg, which indicates that CMV-IVIg do not prevent primary infection but slows down viral replication. This is supported by a decrease in protein synthesis and viral replication of CMV-infected cells *in vitro* in the presence of CMV-IVIg [18], which may be a direct result of the neutralizing activity of immunoglobulins. Interestingly, we do not have any evidence that this lower replicative activity in patients managed with CMV-IVIg adversely affects induction of CMV-specific T cells. In support of this observation, CMV-IVIg have recently been shown to induce CMV-specific T-cell activation, potentially mediated by a more efficient uptake and processing of viral antigens by antigen-presenting cells in the presence of CMV-specific immunoglobulins [19]. From an immunological point of view, prophylaxis with CMV-IVIg may contribute towards control of viral replication and clinical symptoms while still allowing some extent of replication to facilitate induction of specific immunity. This may be different from complete suppression of viral replication during prophylaxis with antiviral drugs such as letermovir or VGCV, where primary infection and induction of specific T cells has been shown to be generally delayed until prophylaxis is discontinued [15,20]. Based on the half-life of CMV-IVIg of 25 days [21], the effect of CMV-IVIg may extend well beyond its last application. The importance of inducing a sufficient CMV-specific T-cell response for virus control in the long-term is emphasized by the fact that patients with subsequent CMV reactivation or EOD had lower levels of CMV-specific T cells following treatment of primary infection, which is in line with previous reports [6,22,23].

Our retrospective study design has some inherent limitations, as not all parameters determining clinical outcomes such as polymedication and comorbidities can easily be controlled for. Moreover, despite having retrieved consecutive data from all patients from our center with and

without VGCV-associated leukopenia, with all patients treated and managed according to the same standards, the overall sample size and subgroups managed with and without CMV-IVIg are in part small. A strength of our study is the integration of both viral load and CMV-specific T-cell analyses. This increases our mechanistical insights on the role of immunoglobulins, and may give guidance on using CMV-IVIg in clinical situations where evidence from randomized controlled trials is lacking.

5. Conclusion

Discontinuation of VGCV prophylaxis followed by close monitoring of viral replication appears to be a safe and effective strategy in patients with VGCV-associated leukopenia. In addition, CMV-IVIg at discontinuation of prophylaxis seems to have a protective effect against high-level CMV DNAemia. Future studies with larger sample sizes should address the direct and indirect effects of CMV-IVIg in lowering viral load and inducing CMV-specific T-cell expansions.

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Data sharing statement

All data of this manuscript will be shared upon reasonable request to the corresponding author.

CRediT authorship contribution statement

Amina Abu-Omar: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Janine Mihm:** Writing – review & editing, Validation, Supervision, Methodology, Investigation, Formal analysis, Data curation. **Saskia Bronder:** Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Formal analysis. **Tina Schmidt:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Martina Sester:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Urban Sester:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.trim.2025.102188.

Data availability

Data will be made available on request.

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