



Full Length Article

Allogeneic - Adult

Association of Cyclosporine Exposure Post-Allogeneic Hematopoietic Cell Transplant With Graft Failure and Relapse Risk in Hematological Malignancies

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A B S T R A C T

Background: Allogeneic hematopoietic cell transplantation (HCT) is a cornerstone in the treatment of many high-risk hematological malignancies. Calcineurin inhibitors (CNIs), essential components of GVHD prophylaxis, require careful monitoring of levels to optimize outcomes. This study evaluated the association between cyclosporine (CsA) exposure during the first 90 days post-HCT and key transplant outcomes.

Methods: A retrospective analysis was performed on 373 patients who underwent allogeneic HCT between January 2019 and July 2021 at the Princess Margaret Cancer Centre. CsA trough levels were routinely measured, and area under the curve for the first 90 days post-transplant (AUC90) was calculated. Associations between CsA AUC90 and graft failure (GF), relapse, acute and chronic GVHD (aGVHD, cGVHD), and infections were assessed.

Results: In a cohort predominantly receiving contemporary GVHD prophylaxis with PTCy-based regimens, higher CsA AUC90 was significantly associated with a reduced risk of GF ($P < .001$) and lower grades of aGVHD ($P < .001$). Univariable analysis confirmed that higher CsA exposure was linked to a lower risk of GF (HR 0.22, $P < .001$). CsA AUC90, however, was not associated with relapse risk ($P = .56$) or cGVHD severity ($P = .38$). Increased CsA exposure was associated with a higher risk of CMV reactivation ($P = .03$), though this risk was mitigated in patients receiving letermovir prophylaxis ($P = .44$).

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Conclusion: This study underscores the importance of CsA monitoring to reduce the risks of GF and aGVHD without increasing relapse in hematological malignancies. However, higher CsA exposure requires careful management due to its association with CMV reactivation. These findings contribute to optimizing immunosuppression strategies in the context of contemporary GVHD prophylaxis.

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INTRODUCTION

Allogeneic hematopoietic cell transplantation (HCT) remains a pivotal therapeutic intervention for numerous hematological malignancies and disorders [1]. However, graft-versus-host disease (GVHD) continues to be a major contributor to post-transplant morbidity and a significant determinant of non-relapse mortality [2]. Consequently, optimizing GVHD prophylaxis regimens is a critical aspect of the complex therapeutic framework involved in allogeneic HCT [3].

Calcineurin inhibitors (CNIs), such as cyclosporine (CsA), have been a fundamental component of GVHD prophylaxis regimens following allogeneic HCT for decades [4]. CNIs exert their immunosuppressive effects by inhibiting calcineurin, a crucial enzyme involved in T-cell activation. This inhibition prevents the dephosphorylation and nuclear translocation of the nuclear factor of activated T cells (NFAT), subsequently suppressing the production of cytokines, including interleukin-2 (IL-2), interferon-gamma (IFN- γ), and tumor necrosis factor-alpha (TNF- α), which are critical for T-cell proliferation and activation [5,6].

Immunosuppressive agents, although primarily utilized as essential components of GVHD prophylaxis regimens, have been shown to exert effects beyond GVHD prevention, influencing critical outcomes such as engraftment success, the risk of graft rejection, and the likelihood of disease relapse [7–11].

Despite their extensive use, robust data linking specific CNI levels to transplant outcomes remain limited. Nevertheless, several studies have investigated the association between targeted CNI levels, particularly CsA, and clinical outcomes in patients undergoing allogeneic HCT. Bianchi et al. demonstrated that CsA levels exceeding 195 $\mu\text{g/L}$ on day 10 post-transplant were significantly correlated with a reduced incidence of acute GVHD (aGVHD). Their cohort predominantly received peripheral blood stem cell grafts (89%), myeloablative conditioning (78.6%), and GVHD prophylaxis with CsA plus methotrexate (65.2%), with

anti-thymocyte globulin (ATG) administered in 30% of cases [12]. Similarly, Nikoloudis et al. reported that CsA levels above 200 ng/mL during the early post-transplant period were associated with a lower risk of grade 3–4 aGVHD, without negatively impacting relapse rates. This study excluded post-transplant cyclophosphamide (PTCy)-based regimens, used anti-T-lymphocyte globulin-based T-cell depletion in 71.7% of patients, and relied on methotrexate (MTX)- (57.7%) or mycophenolate mofetil (MMF)-based (42.3%) GVHD prophylaxis [13].

Earlier studies have also explored the impact of CsA starting dose regimens on transplant outcomes. Bacigalupo et al. compared high- versus low-dose CsA (5 vs. 1 mg/kg/day) and found reduced aGVHD with high-dose CsA, but a higher relapse rate (52% vs. 20%; $P = .001$) [14,15]. A pediatric study similarly demonstrated that early high-dose CsA was associated with increased relapse risk [8]. However, both studies focused on initial dosing rather than cumulative exposure.

Previous studies have primarily focused on evaluating median CsA levels, starting doses, or occasionally the area under the curve (AUC), often restricted to CsA exposure during the first month post-transplant [14,16–18]. Similar methodological limitations apply to studies assessing tacrolimus concentrations, which have likewise focused on short-term exposure and early post-transplant levels [19,20].

In this study, we aim to provide a comprehensive analysis of cumulative CsA exposure over an extended post-transplant period and its potential impact on key transplant outcomes. In addition to GVHD, we explored the associations of CsA exposure with graft failure, relapse, CMV/EBV reactivation, and overall survival. By addressing the balance between implementing an effective GVHD prevention strategy and minimizing relapse risk, our findings seek to inform clinical practice, particularly in settings where target CsA levels vary based on patient-specific factors and institutional protocols. This study focused on

patients receiving various GVHD prophylaxis regimens, the majority of whom were treated with contemporary PTCy-based approaches, most commonly PTCy combined with ATG, in both matched and mismatched donor transplant settings. We hypothesized that higher CsA exposure during the early post-transplant period would be associated with a reduced incidence of acute GVHD and a potentially increased risk of relapse.

METHODS

Study Design and Patient Selection Criteria

We conducted a retrospective analysis of 373 patients who underwent allogeneic HCT at the Princess Margaret Cancer Centre, University Health Network, Toronto, Canada, between January 1, 2019, and July 31, 2021. As part of routine post-transplant care, CsA trough levels were monitored at specified intervals—twice weekly during the first 60 days post-transplant and subsequently approximately once weekly until CsA discontinuation. CsA exposure was quantified by calculating the AUC over the first 90 days post-transplant, and its association with various transplant outcomes was analyzed.

Patients with non-malignant hematologic conditions, those receiving bone marrow or cord blood as the graft source, and those who discontinued CsA within the first 30 days post-transplant were excluded from the analysis. This study was approved by the University Health Network Research Ethics Board and the Cancer Registry Data Access Committee and was conducted in accordance with the principles outlined in the Declaration of Helsinki.

Variables

Data collected included patient demographics, such as age and sex, as well as clinical variables including transplant indications, hematopoietic cell transplantation comorbidity index (HCT-CI), Karnofsky performance status (KPS), disease risk index (DRI), donor type and age, CD34+ cell dose, conditioning regimen intensity, and GVHD prophylaxis regimen. Post-transplant events, including relapse, graft failure (GF), acute and chronic graft-versus-host disease (aGVHD, cGVHD), cytomegalovirus (CMV) reactivation (defined as CMV PCR >200 IU/mL), Epstein-Barr virus (EBV) reactivation (defined as EBV PCR >1 500 IU/mL), and bloodstream infections (BSI), were recorded. For patients developing aGVHD, the CsA AUC was calculated up to the onset of aGVHD to assess its correlation.

Conditioning Regimen

The conditioning regimens for patients undergoing allogeneic HCT were categorized as reduced-intensity conditioning (RIC) or myeloablative conditioning (MAC) as per internationally accepted definitions. RIC regimens included several variations: fludarabine at 35 mg/m² daily for 4 days alongside treosulfan at either 10 g/m² or 14 g/m² daily for 3 days, or fludarabine at 35 mg/m² daily for 4 days (starting on day-5) with busulfan at 3.2 mg/kg daily for 2 days (starting on day-5), with TBI of 2 Gy on day-1. MAC regimens also involved different combinations, including fludarabine at 35 mg/m² daily for 4 days (starting on day-5) with busulfan at 3.2 mg/kg daily for 4 days (starting on day-5); fludarabine at 40 mg/m² daily for 3 days (starting on day-5) with TBI of 2 Gy administered twice daily for 3 days (starting on day-3); and etoposide at 60 mg/kg administered as a single dose on day-5 combined with TBI of 2 Gy delivered twice daily for 3 days (starting on day-3).

GVHD Prophylaxis

GVHD prophylaxis included any of the following regimens: (1) anti-thymocyte globulin (ATG) administered either at a total dose of 4.5 mg/kg on days -3, -2, and -1 or 2 mg/kg on days -2 and -1, combined with post-transplant cyclophosphamide (PTCy) at 50 mg/kg for 2 days starting on day +3 and cyclosporine A (CsA) at 2.5 mg/kg every 12 hours (q12h) starting on day +5; (2) PTCy at 50 mg/kg for 2 days starting on day +3, mycophenolate mofetil (MMF) at 15 mg/kg every 8 hours (q8h) starting on day +5, and CsA at 2.5 mg/kg q12h starting on day +5; (3) ATG at 2 mg/kg combined with methotrexate (MTX) at 15 mg/m² on day +1 and 10 mg/m² on days +3 and +6, with CsA at 2.5 mg/kg q12h starting on day -1; (4) CsA at 2.5 mg/kg q12h starting on day -1 combined with MMF at 15 mg/kg q8h starting on day +1; (5) CsA at 2.5 mg/kg q12h starting on day -1 combined with MTX at 15 mg/m² on day +1 and 10 mg/m² on days +3 and +6. At our institution, the standard GVHD prophylaxis includes PTCy-based regimens: ATG+PTCy+CsA for matched unrelated donors (ATG 2 mg/kg) and haploidentical donors (ATG 4.5 mg/kg), and PTCy+MMF+CsA for matched related donors.

Infectious Prophylaxis

Infectious prophylaxis consisted of ciprofloxacin 500 mg orally once daily from day -6 until engraftment, alongside either micafungin 50 mg IV daily or caspofungin (70 mg IV as a loading

dose, then 50 mg IV daily) starting on day +1 until engraftment. For antiviral prophylaxis, valacyclovir 500 mg twice daily was initiated on day +1 and continued for up to 1-year post-transplant. Posaconazole 300 mg orally once daily was started at engraftment and maintained until approximately day +100. PJP prophylaxis was introduced at engraftment or day +28 and continued for 1 year. From January 2020, letermovir was prescribed daily from day +21 for 100 days for patients considered high risk for CMV reactivation (CMV seropositive patients receiving ATG).

Statistical Methods, Endpoints, and Analysis

The primary outcomes of interest were cumulative incidence of aGVHD, GF, and cumulative incidence of relapse (CIR). Secondary outcomes included cumulative incidence of cGVHD, post-transplant infections, overall survival (OS; defined from the time of transplant until death or last follow up) and non-relapse mortality (NRM). Acute and chronic GVHD were graded according to established criteria.

The linear trapezoidal method, which relies on linear interpolation between data points, was employed to calculate the area under the curve (AUC). For a given time interval (t_1-t_2), the AUC is calculated using the formula:

$$AUC = 1/2(C_1 + C_2)(t_2 - t_1)$$

Here, the first term represents the average drug concentration over the interval, while the second term (t_1-t_2) accounts for the duration of the interval. By multiplying the average concentration with the time interval and summing these values across all intervals, the total drug exposure from the first to the last time point is obtained. The average concentration between each pair of measurements was computed using this method, and the total AUC90 was derived by summing all interval AUCs up to day +90.

Kaplan-Meier and log-rank analysis was used to evaluate OS. CIR, NRM, aGVHD incidence, cGVHD incidence, CMV and EBV reactivations were calculated using competing risk regression. For NRM and CIR, relapse or death in the absence of relapse were the competing event, respectively. For cumulative incidence of aGVHD, cGVHD, CMV and EBV reactivation, death (within 100d for aGVHD) was the competing event. For cGVHD only patients surviving more than 100 days were included in the analysis. A 2-sided P-value of $\leq .05$ was considered statistically significant. Categorical variables were compared using the χ^2 test or Fisher's exact test (depending on the number

of data entrances). Descriptive statistics were used to report clinical characteristics. Multivariate survival analysis was performed with the Cox regression model and Fine and Gray, adjusting for independent variables. Statistical analysis was conducted using Statistica software, version 14.2 (TIBCO, Palo Alto, CA, USA) and EZR version 1.61 (freely available software) [21].

RESULTS

Baseline Characteristics

A total of 373 patients underwent allogeneic HCT, with a median age of 58 years (range 18-76); 54.2% were male. The primary diagnoses included acute leukemia (65%), MDS/MPN (25.9%), lymphoma (5.1%), and chronic leukemia (4%). Most patients (56.6%) were transplanted in CR1, with a median CD34+ cell dose of 7.3×10^6 cells/kg (range 0.3-19.4). Donors included matched unrelated donor (MUD) (48.5%), matched related donor (MRD) (22.3%), haploidentical (19.0%), and mismatched unrelated donor (MMUD) (10.2%). Conditioning regimens were predominantly RIC (59.5%), with 20.9% receiving frozen grafts and 34.3% receiving letermovir CMV prophylaxis. GVHD prophylaxis included ATG alone (16.4%), PTCy alone (12.9%), or PTCy + ATG (69.4%), with 66.5% receiving 2 mg/kg ATG. Most patients had a KPS ≥ 90 (83.5%) and an HCT-CI < 3 (65.9%).

Table 1

CsA AUC Analysis

The descriptive statistics for AUC90, reflecting cumulative cyclosporine A exposure over the first 90 days post-transplant, showed a mean of 18 382 ng·h/mL, with a median of 18 364 ng·h/mL.

When stratified by diagnosis, the mean AUC90 for chronic leukemia was approximately 20,000 ng·h/mL, compared to around 18 000 ng·h/mL for acute leukemia, lymphoma, and MDS/MPN ($P = .04$).

Analysis of donor type, including haploidentical, MMUD, MRD, and MUD, showed mean AUC90 values ranging from 17 000 to 19,000 ng·h/mL ($P = .61$). For conditioning regimens, patients receiving MAC had a mean AUC90 of approximately 19,000 ng·h/mL, while those receiving RIC had a mean of around 18,000 ng·h/mL ($P = .19$). Regarding GVHD prophylaxis, the mean AUC90 for patients receiving ATG alone was approximately 20 000 ng·h/mL, while those receiving PTCy or a combination of PTCy and ATG had mean values near 18 000 ng·h/mL ($P = .42$).

Table 1
Baseline Patient and Transplant Characteristics

| Characteristics | Results |
|-----------------------|----------------|
| No. | 373 |
| Age | 58 (18-76) |
| Sex | |
| Male | 202 (54.2%) |
| Female | 171 (45.8%) |
| Diagnose | |
| AcL | 241 (65%) |
| ChL | 15 (4%) |
| MDS/MPN | 96 (25.9%) |
| Lymphoma | 19 (5.1%) |
| Stage | |
| CR1 | 211(56.6%) |
| CR2-3 | 43 (11.5%) |
| KPS <90 | 59 (16.5%) |
| HCT-CI ≥ 3 | 122 (34.1%) |
| CD34 dose | 7.3 (0.3-19.4) |
| Donor Type | |
| MRD | 83 (22.3%) |
| MUD | 181 (48.5%) |
| HAPLOIDENTICAL | 71 (19%) |
| MMUD | 38 (10.2%) |
| Frozen Graft | 78 (20.9%) |
| Conditioning | |
| RIC | 222 (59.5%) |
| MAC | 151 (40.5%) |
| Letemovir prophylaxis | 128 (34.3%) |
| GVHD prophylaxis | |
| ATG | 61(16.4%) |
| PTCy | 48 (12.9%) |
| PTCy+ATG | 259 (69.4%) |
| Other | 5 (1.3%) |
| ATG dose | |
| 2 mg/kg | 248 (66.5%) |
| 4.5 mg/kg | 71 (19%) |
| DRI | |
| Low | 17 (4.6%) |
| IM | 297 (80.3%) |
| High | 56 (15.1%) |

AcL, acute leukemia; ATG, anti-thymocyte globulin; ChL, chronic leukemia; CR1, first complete remission; CR2-3, second/third complete remission; DRI, disease risk index; GVHD, graft-versus-host disease; HCT-CI, hematopoietic cell transplant comorbidity index; IM, intermediate risk; KPS, Karnofsky performance status; MAC, myeloablative conditioning; MDS/MPN, myelodysplastic syndrome/myeloproliferative neoplasm; MMUD, mismatched unrelated donor; MRD, matched related donor; MUD, matched unrelated donor; PTCy, post-transplant cyclophosphamide; RIC, reduced intensity conditioning.

When analyzed based on DRI, patients classified as low risk exhibited a mean AUC90 of approximately 20,000 ng·h/mL, while those in the intermediate and high-risk categories demonstrated mean values around 18 000 ng·h/mL ($P = .72$).

Graft Failure

The total number of patients that experienced GF was 16 (4.3%), primary GF in 7 patients (1.9%) and secondary GF in 9 patients (2.4%). The analysis of CsA AUC90 revealed lower values in patients with GF compared to those without (mean AUC90 ~ 12 000 ng·h/mL vs. ~ 18 000 ng·h/mL, $P < .001$). Primary GF was associated with lower AUC90 compared to secondary GF and no GF ($P = .004$) (Figure 1A,1B).

Univariable analysis identified CsA AUC90 as a continuous variable (HR 0.22; 95% CI 0.06-0.76, $P < .001$) and CsA AUC90 above the median (HR 0.22; 95% CI 0.06-0.76, $P = .017$) as being associated with a lower risk of GF. Risk factors for GF included myelofibrosis (HR 5.81; 95% CI 2.02-16.7, $P = .001$) and frozen grafts (HR 5.22; 95% CI 1.94-14.0, $P = .001$).

Multivariable analysis confirmed myelofibrosis (HR 6.18; 95% CI 2.14-17.83, $P < .001$) and frozen grafts (HR 5.44; 95% CI 2.02-14.63, $P < .001$) as independent risk factors for GF. CsA AUC90 as a continuous variable was associated with a lower risk for GF (HR 0.99; 95% CI 0.99-1.00, $P = .002$). Longitudinal CsA level analysis revealed significantly lower levels in patients with GF compared to those without GF. Differences were observed at weeks 3 ($P = .002$), 5 ($P = .035$), and 8 ($P = .01$) (Figure 1C).

In addition, we compared the mean duration of CsA administration between patients who developed GF and those who did not and found no statistically significant difference (101 vs. 110 days, $P = .13$), suggesting that early discontinuation of CsA was unlikely to have contributed to the lower AUC observed in graft failure cases.

Relapse and CsA Exposure

The analysis of CsA AUC90 showed no significant difference between patients without relapse and those that relapsed beyond the initial 90 days ($P = .56$) (Figure 2A). However, patients who relapsed within the initial 90 days had lower mean AUC90 compared to those relapsing later ($P = .024$), most likely because of prompt discontinuation of immunosuppression upon diagnosis of relapse (Figure 2B).

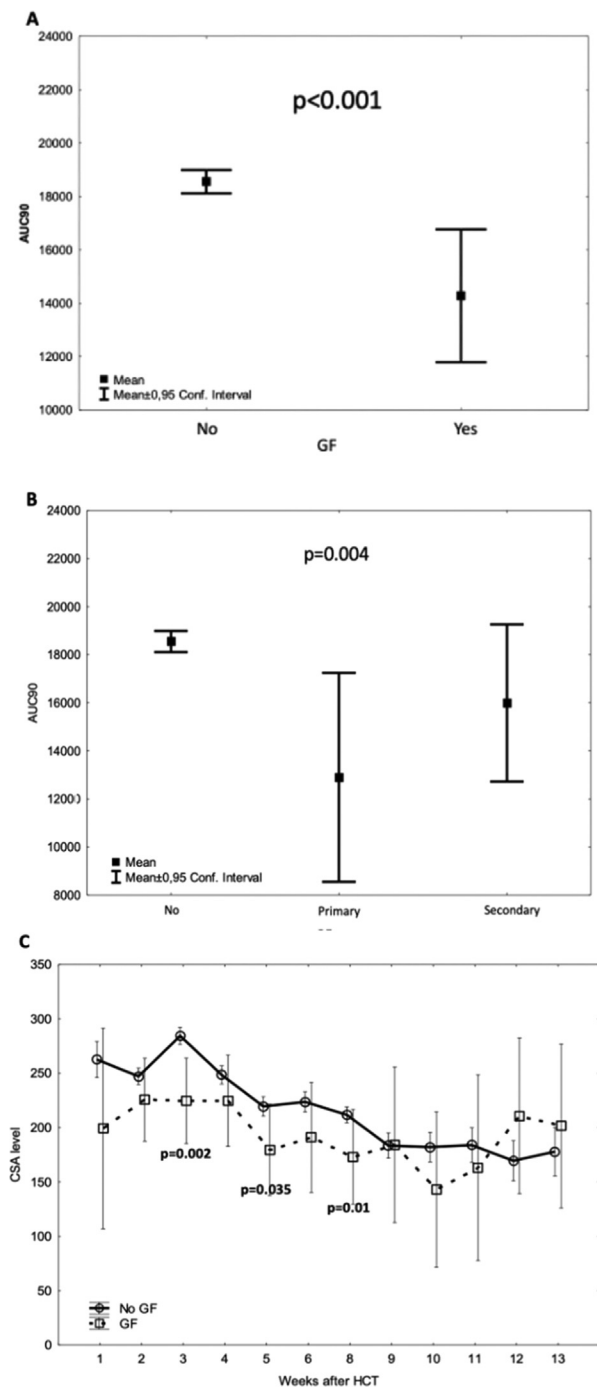


Figure 1. Graft failure association with CsA AUC90. (A) Graft failure (GF) and CsA AUC90, (B) Type of graft failure (primary vs secondary) and CsA AUC90, (C) CsA level correlation with graft failure.

To further investigate this, we compared the mean duration of CsA administration between patients who experienced relapse and those who did not. No significant difference in immunosuppression duration was observed (102 vs. 112 days, $P = .64$).

In Fine and Gray competing risks analysis, high DRI was strongly associated with relapse (HR

2.94; 95% CI 1.91–4.52, $P < .001$), while MAC showed a trend toward reducing relapse risk (HR 0.67; 95% CI 0.44–1.01, $P = .05$).

Graft-Versus-Host Disease

In our cohort, the cumulative incidence of aGVHD grade III–IV was 8.6% (95% CI 6.0–11.7) at 100 days and 12.2% (95% CI 9.1–15.7) at 1 year. The cumulative incidence of chronic GVHD (cGVHD) was 34.0% (95% CI 29.0–39.0) at 1 year and 30.0% (95% CI 33.9–44.2) at 3 years.

Analysis of CsA AUC, prior to the onset of aGVHD at any time, demonstrated a significant association between AUC values and aGVHD severity, with lower mean AUC values observed as aGVHD grade increased ($P < .001$) (figure 3A). This correlation between lower AUC and increased aGVHD severity was consistent across all donor types in our cohort: MUD ($P = .003$), haploidentical ($P = .003$), MRD ($P < .001$), and MMUD ($P = .001$) (Supplementary Figure 1). Regarding cGVHD, no significant differences in CsA AUC90 were observed across severity categories (mild, moderate, severe; $P = .38$) (Figure 3B).

To further explore the CsA exposure threshold associated with lower aGVHD risk, we stratified patients based on mean CsA levels prior to aGVHD onset. A mean value above 205 ng/mL was associated with a significantly reduced incidence of grade II–IV aGVHD (18.7% vs. 29.5%, $P = .01$) (Supplementary Figure 2).

Univariable analysis (UVA) revealed associations between CsA AUC prior to aGVHD (HR 0.99; 95% CI 0.99–0.99, $P < .001$), donor age (HR 1.01; 95% CI 1.00–1.02, $P = .038$), and PTCy-based GVHD prophylaxis (HR 0.65; 95% CI 0.44–0.96, $P = .03$) with the risk of aGVHD grade II–IV. Multivariable analysis (MVA) identified donor age (HR 1.01; 95% CI 1.00–1.02, $P = .03$) and PTCy (HR 0.62; 95% CI 0.42–0.92, $P = .02$) as independent predictors of aGVHD grade II–IV.

Survival and Post-Transplant Infections

The OS in our cohort at 100 days, 1 year, and 3 years post-HCT was 92.2% (95% CI 89.0–94.5), 77.5% (95% CI 72.9–81.4), and 63.9% (95% CI 58.8–68.6), respectively. There was no significant association between CsA AUC90 and overall survival ($P = .99$) (Supplementary Figure 3). The cumulative incidence of non-relapse mortality (NRM) was 6.2% (95% CI 4.2–9.2) at 100 days and 13.9% (95% CI 10.6–17.9) at 1 year.

Patients with CMV reactivation had higher mean AUC90 levels compared to those without reactivation ($P = .03$). In patients not receiving

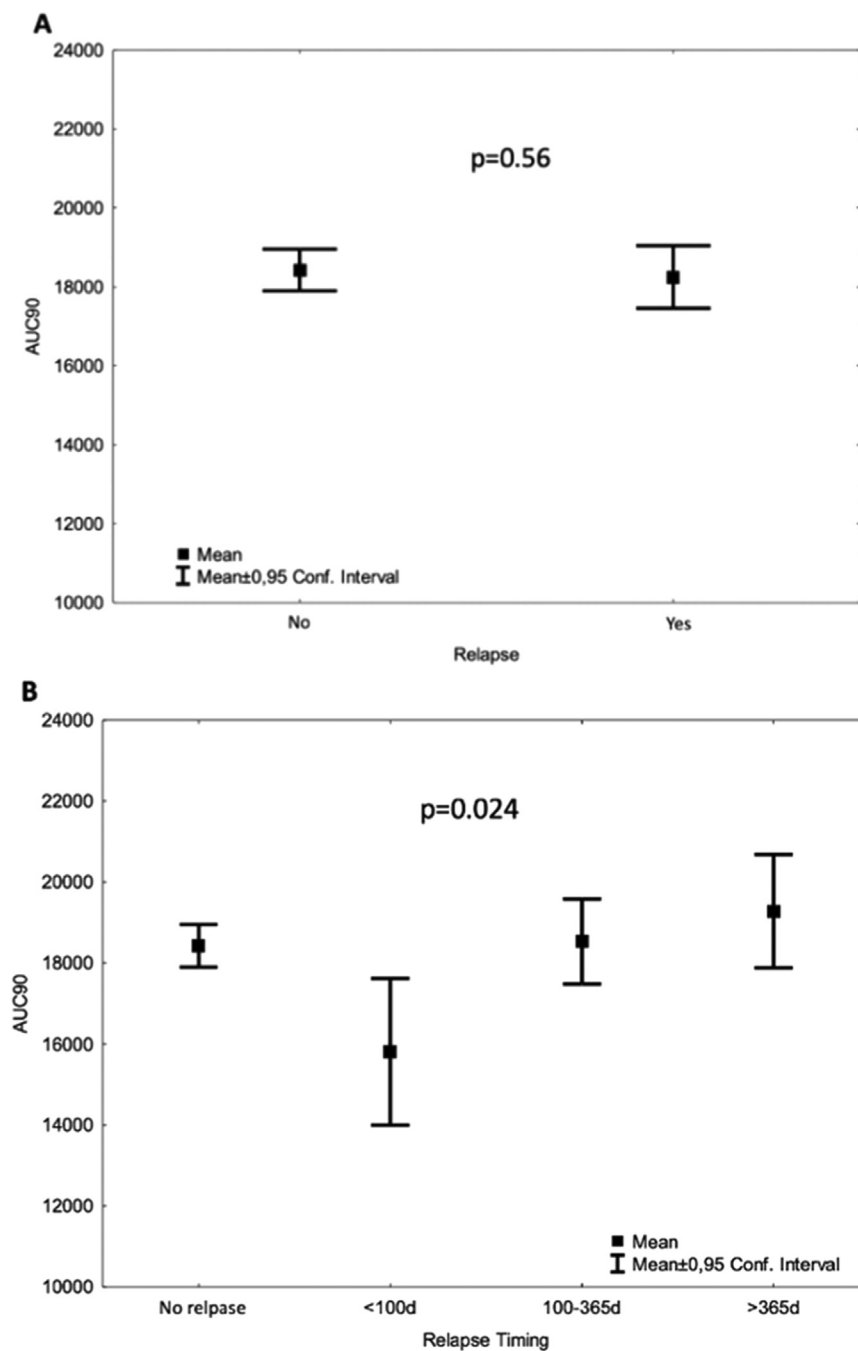


Figure 2. Association between CsA AUC90 and disease relapse. (A) Relapse incidence and CsA AUC90, (B) Relapse timing and CsA AUC90.

letermovir prophylaxis, CMV reactivation was associated with higher AUC90 levels ($P = .035$), whereas no significant difference was observed in the letermovir group ($P = .44$) (Figure 4A,4B,4C).

Further analysis based on the number of CMV infections demonstrated a higher recurrence rate in patients with higher AUC90 levels ($P = .027$). This trend was more evident in the non-letermovir group ($P = .06$) compared to the letermovir group ($P = .44$) (Figure 5A,5B,5C).

MVA identified HLA mismatch (HR 1.88; 95% CI 1.37-2.57, $P < .001$), CD34 dose (HR 1.12; 95% CI 1.05-1.19, $P < .001$), and age at transplant (HR 1.02; 95% CI 1.01-1.03, $P < .001$) as independent predictors of CMV reactivation.

EBV reactivation showed no significant difference in mean AUC90 levels between patients with and without reactivation ($P = .23$). Similarly, no significant association was observed between AUC90 levels and the timing of bloodstream

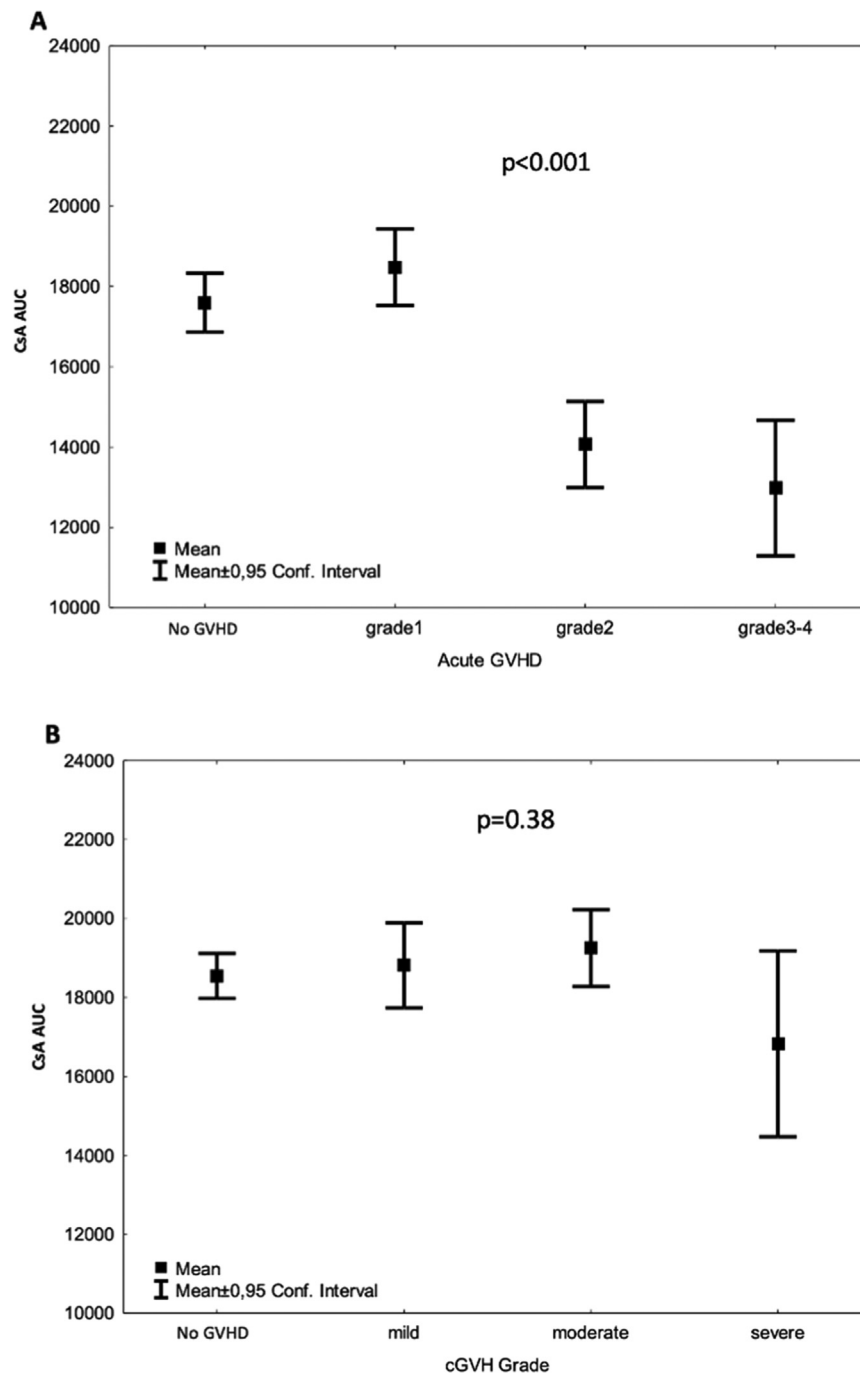


Figure 3. GVHD severity and correlation with CsA AUC. (A) Acute GVHD severity and CsA AUC (calculated up to the onset of aGVHD), (B) Chronic GVHD severity and CsA AUC90.

infections (<30 days, 30-100 days, or >100 days post-HCT; $P = .17$) (supplementary figure 4).

ATG + PTCy Subgroup Analysis

Given that the majority of patients (69.4%) received GVHD prophylaxis with a combination of ATG and PTCy, we performed a subgroup analysis restricted to this population to reduce heterogeneity in GVHD prophylaxis protocols. The associations between CsA AUC90 and key clinical

outcomes—including acute GVHD, graft failure, and relapse—were preserved in this subgroup (supplementary figure 5).

DISCUSSION

This study provides a comprehensive evaluation of CsA exposure during the initial 90 days following allogeneic HCT in a large patient cohort. The findings highlight a significant association between CsA exposure, measured as AUC90, and

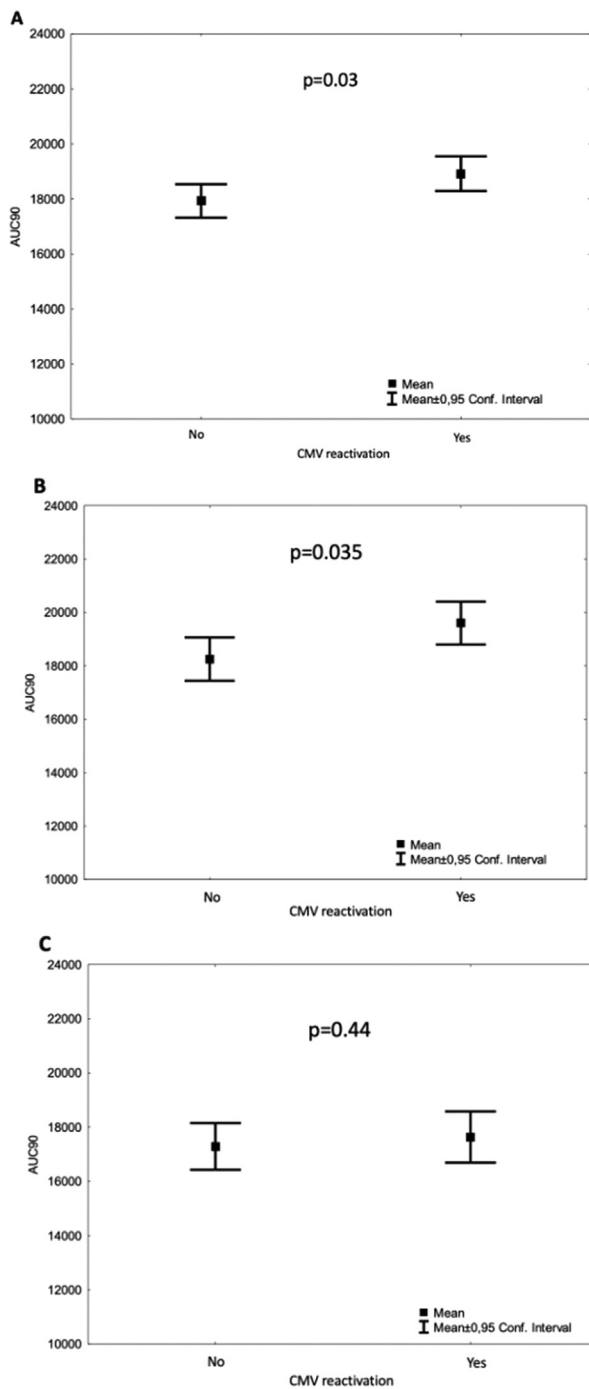


Figure 4. Association between CsA AUC90 and CMV reactivation. (A) CsA AUC90 and CMV reactivation in the entire cohort, (B) CsA AUC90 and CMV reactivation in patients not receiving letermovir prophylaxis, (C) CsA AUC90 and CMV reactivation in patients receiving letermovir prophylaxis.

several key post-transplant outcomes. Our analysis offers novel insights into the relationship between immunosuppressive drug levels and clinical outcomes, contributing to the growing evidence base for optimizing immunosuppressive strategies in the post-HCT setting.

In our cohort, the majority of patients received GVHD prophylaxis with a PTCy-based regimen,

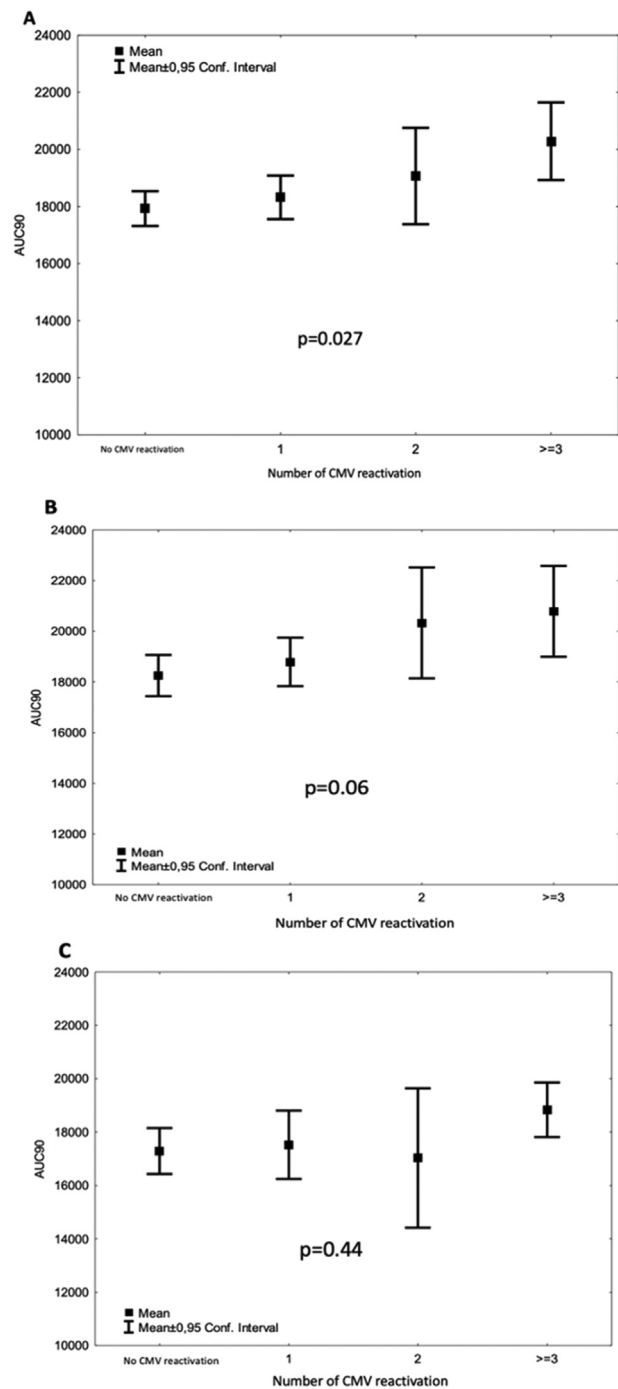


Figure 5. Association between CsA AUC90 and recurrent CMV reactivation. (A) CsA AUC90 and CMV reactivation recurrence in the entire cohort, (B) CsA AUC90 and CMV reactivation recurrence in patients not receiving letermovir prophylaxis, (C) CsA AUC90 and CMV reactivation recurrence in patients receiving letermovir prophylaxis

predominantly a combination of ATG and PTCy (69.4%). While variations in GVHD prophylaxis strategies were present, no significant differences in CsA exposure levels were observed across the different regimens ($P = .42$).

Craddock et al. conducted a detailed analysis of CsA exposure by evaluating AUC over the first

21 days post-HCT. Their results demonstrated that increased CsA exposure was associated with a higher risk of relapse (HR 1.83; $P < .001$) [18]. However, this analysis was limited by a small sample size of 63 patients, all of whom received a reduced-intensity conditioning regimen with alemtuzumab-based GVHD prophylaxis.

In our study, intriguingly, no association was observed between CsA AUC90 and an increased risk of relapse. Furthermore, when relapse was stratified by timing, patients who experienced early relapse had lower mean CsA AUC90 levels ($P = .024$), however this finding most likely reflects the early tapering of CsA following the diagnosis of relapse. Among the cohort, 59.5% received RIC and 40.5% underwent MAC, with no significant differences in CsA AUC90 identified between these groups ($P = .19$).

The graft-versus-tumor (GVT) effect is a fundamental component of allo-HCT in hematological malignancies, leveraging alloreactive donor T cells to prevent disease relapse by targeting residual malignant cells [22,23]. However, the clinical challenge lies in balancing immunosuppression to mitigate the risk of GVHD while preserving the beneficial GVT effect. Previous studies have evaluated the impact of in vivo T-cell depletion using ATG. While 1 report raised a safety concern, many prospective studies have not demonstrated an increased risk of relapse associated with its use [24–27]. Similarly, prior analyses have reported no significant difference in relapse incidence between patients receiving PTCy alone and those receiving a combination of ATG and PTCy [28–30].

One of the significant findings of our study is the association between lower CsA AUC90 and an increased risk of GF ($P < .001$). This highlights the importance of maintaining adequate CsA exposure during the early post-transplant period to support engraftment, even in patients receiving potent GVHD prophylaxis regimens incorporating PTCy and ATG. Minimizing the risk of GF is particularly essential in the context of haploidentical and mismatched unrelated donor transplants, where the inherent risk of GF is significantly higher [31,32].

Our report further highlights the role of CsA exposure in the prevention of aGVHD. A significant inverse relationship was identified between CsA AUC90 and aGVHD severity, with lower CsA levels preceding the onset of higher-grade aGVHD (grade III-IV). This association was consistent across all donor types, including MUD, haploidentical donors, MRD, and MMUD. In contrast, no

significant association was found between CsA AUC90 and the severity of cGVHD, suggesting that the impact CsA and mechanisms driving cGVHD differ from those influencing aGVHD [33]. We compare our results to another recent retrospective study which investigated the impact of tacrolimus and sirolimus levels during the first 4 weeks post-transplant on outcomes of patients transplanted for hematologic malignancies and that received PTCy prophylaxis [34]. Weekly average levels of tacrolimus and sirolimus were investigated during the first 4 weeks post-transplant, as opposed to our method of assessment of AUC90. The study found no impact on cumulative incidence of aGVHD, while higher levels of sirolimus and tacrolimus were correlated with decreased OS. Similar to our study, no impact of levels of both drugs was seen on relapse-free survival. Of note, the majority of patients in that cohort receiving tacrolimus had received bone marrow grafts, while all patients had received as conditioning a combination of fludarabine, cyclophosphamide and TBI [34].

Our study is the first to comprehensively evaluate CsA exposure during the initial 90 days post-hematopoietic cell transplantation in a cohort receiving predominantly PTCy-based prophylaxis. While AUC remains the most precise pharmacokinetic summary of systemic drug exposure, translating AUC to actionable trough levels remains challenging and requires further validation. In our cohort, mean CsA levels greater than 205 ng/mL were associated with a lower incidence of severe aGVHD. These data suggest that maintaining adequate CsA exposure and avoiding intentional lowering of trough targets out of relapse concern, may help prevent severe aGVHD. We do not advocate exceeding standard CsA targets, but rather emphasize the potential harm of subtherapeutic immunosuppression.

However, this study has several limitations. Its retrospective design introduces potential biases, including unmeasured confounders and variability in clinical practice. The exclusion of patients with non-malignant hematologic conditions limits the applicability of finding to those populations. However, this exclusion was intentional, as the primary focus was to evaluate relapse risk, which is less relevant in non-malignant settings. While the inclusion of diverse donor types and hematologic malignancies improves generalizability, relapse risk may differ by disease type. Additionally, heterogeneity in conditioning intensity, GVHD prophylaxis, and donor types may influence outcomes, particularly GVHD. The

predominance of PTCy-based GVHD prophylaxis in our cohort may also limit generalizability to settings using conventional MTX- or MMF-based regimens.

In conclusion, in patients receiving contemporary GVHD prophylaxis, optimizing CsA exposure is associated with a reduced risk of GF and severe aGVHD without increasing the risk of relapse in hematological malignancies. These findings contribute novel insights into the field and provide a foundation for future research exploring the role of calcineurin inhibitors in patients receiving modern GVHD prophylaxis strategies.

DATA AVAILABILITY STATEMENT

Due to our institutional privacy policy, our data is not publicly available.

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SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at [doi:10.1016/j.jct.2025.05.024](https://doi.org/10.1016/j.jct.2025.05.024).

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