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Cytomegalovirus (CMV) management in allogeneic hematopoietic cell transplantation: Pre-transplant predictors of survival, reactivation and spontaneous clearance

Running Title: Cytomegalovirus management in allogeneic HCT

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AUTHOR CONTRIBUTIONS

JL participated in research design, data collection, statistical analysis and wrote the manuscript; JO participated in data collection, statistical analysis and contributed to the writing and review of the manuscript; IK and WS provided clinical care and contributed to the writing and review of the manuscript; KF and CA provided clinical care and contributed to the review of the manuscript; SC, DK, SP, CL and MS participated in research design and contributed to the writing and review of the manuscript; MG participated in research design, provided clinical care and contributed to the writing and review of the manuscript.

ABSTRACT

Cytomegalovirus (CMV) reactivation is a frequent complication after allogeneic hematopoietic cell transplant (alloHCT). We analyzed 159 alloHCT recipients with 4409 quantitative CMV viral loads to determine pre-transplant predictors of CMV reactivation, clinically significant CMV infection (cs-CMVi, defined as CMV viral load >1000 IU/mL), CMV disease, kinetics of spontaneous clearance of CMV and survival using a standardised pre-emptive therapy approach to identify at-risk groups to target prevention strategies. Cs-CMVi was most common in D-/R+ unrelated donor transplants (URD). Spontaneous CMV clearance occurred in 26% of patients who reached a viral load of 56-137 IU/mL, 6% at 138-250 IU/mL and in 1 patient >250 IU/mL. Median time between the first CMV reactivation (>56 IU/mL) and a viral load >250 IU/mL was 13 days, whereas the time from the first viral load >250 IU/mL to reach a vial load >1000 IU/mL was 4 days. Cs-CMVi was associated with a significant increase in non-relapse mortality (NRM) on multivariate analysis. Overall, this study indicates that D-/R+URD recipients are at high-risk for cs-CMVi and CMV related mortality, and are potential candidates for targeted CMV prophylaxis. Spontaneous clearance of CMV beyond a viral load of 250 IU/mL is uncommon, suggesting that this could be used as an appropriate threshold to initiate pre-emptive therapy.

KEYWORDS

Hematopoietic cell transplantation (HCT), Cytomegalovirus (CMV), Viremia, Pre-emptive therapy

Word Count

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INTRODUCTION

Cytomegalovirus (CMV) reactivation is a frequent complication after allogeneic hematopoietic cell transplant (alloHCT).[1] Transplant recipients who are seropositive (IgG) for CMV have significantly higher non-relapse mortality (NRM) and increased risk of death post-transplant.[2] CMV reactivation has been associated with both early and late NRM as well as poorer overall survival (OS) despite the increasing adoption of pre-emptive therapy.[1,3-5] Some reports have suggested that CMV infection may reduce relapse (particularly in acute myeloid leukemia (AML)[6-8] but this effect was not seen in a recent large multicenter study and remains controversial.[1]

The choice of anti-CMV agent is dependent on efficacy, toxicity, cost, drug-drug interactions as well as ease of administration and vascular access.[9,10] Given potential myelosuppression associated with the use of ganciclovir or valganciclovir as CMV prophylaxis,[11-13] historically most alloHCT centers have adopted a pre-emptive CMV strategy, which has reduced incidence of early CMV disease to <5%.[14,15] Despite this, there is no consensus regarding the frequency of CMV monitoring or viral loads at which pre-emptive therapy should be initiated,[2,3,12,16-18] with institutional thresholds ranging from any detectable level

to >2000 IU/ml.[19,20] More recently, the adoption of the cytomegalovirus terminase inhibitor, letermovir, as CMV prophylaxis has become standard of care in many institutions.[21-23] However, the cost of using letermovir prophylaxis for all alloHCT recipients may be prohibitive.[24]

An optimal CMV management strategy could instead involve tailoring prophylactic or pre-emptive therapy approaches based on individual risk for CMV reactivation and mortality from CMV disease.[10] In order to (1) identify the groups at highest risk of CMV reactivation and CMV related mortality, and (2) establish patterns of CMV reactivation that could guide pre-emptive therapy, we investigated the pre-transplant predictors of CMV reactivation, clinically significant CMV infection (cs-CMVi), sponataneous CMV viral clearance kinetics, CMV disease, and survival in adult alloHCT recipients without CMV prophylaxis, using a standardised approach to the initiation of pre-emptive therapy.

MATERIALS AND METHODS

Study design and participants

This was a retrospective cohort study of alloHCT recipients at Royal North Shore Hospital, Sydney, Australia from 2013-2018 inclusive. All consecutive patients undergoing first alloHCT were enrolled; patients who were administered CMV prophylaxis were excluded. All patients were followed until death or at least 1 year post alloHCT. Institutional databases were accessed to determine CMV reactivation, clinically significant CMV infection (cs-CMVi, defined as CMV viral load >1000 IU/mL), CMV disease (defined by the European Conference on Infections in Leukaemia, ECIL 7 criteria)[9,25] and survival at 1-year based on pre-transplant risk factors of CMV serostatus, donor type by matched related donor (MRD), unrelated donor (URD), HLA match of URD including mismatched unrelated donor (MMURD) or matched unrelated donor (MURD), conditioning intensity, primary disease, disease risk index (DRI)[26], standard graft versus host disease (GVHD) prophylaxis, patient age and hematopoietic cell transplant co-morbidity index (HCT-CI)[27]. To exclude the potential confounding effect of acute GVHD (aGVHD) on cs-CMVi or mortality between pre-transplant variables,[21, 22] the incidence of any grade II-IV aGVHD was also assessed. Institutional Human Ethics Committee approval was obtained (HREC/50095/PMCC-2019).

HCT Protocol

The conditioning protocol was determined according to patient and disease characteristics, and categorised as myeloablative (MAC) or reduced intensity (RIC) as previously defined, with non-myeloablative regimens included as RIC.[28] The most commonly used regimes consisted of fludarabine/melphalan (FluMel)[29] for RIC and busulfan/cyclophosphamide (BuCy) with therapeutic drug monitoring or cyclophosphamide/total body irradiation (CyTBI) for MAC. Standard GVHD prophylaxis consisted of cyclosporin and methotrexate (5-15mg/m^2 on days +1, +3, +6 and +11); in addition all URD received either

"Ruutu" protocol[30] corticosteroids (prior to 2015) or Thymoglobulin (ATG) 4.5mg/kg (2015 onwards). The "Ruutu" protocol consisted of methylprednisolone or prednisolone at 0.5 mg/kg on days 14 to 20, 1 mg/kg on days 21 to 34, 0.5 mg/kg on days 35 to 48, and then slowly tapered till day 110. The most common stem cell source was G-CSF mobilised peripheral blood stem cells collected by apheresis (PBSC). All patients were administered anti-infective prophylaxis with valacyclovir 500mg daily from day +1 onwards, sulfamethoxazole 800mg / trimethoprim 160mg (Bactrim DS) BID twice weekly from engraftment onwards, and itraconazole 200mg BID from day +1 until immune suppression was weaned.

CMV serostatus, monitoring and therapy

CMV IgG serology for both donor (D) and recipient (R) were assessed using an Abbot Architect CMV IgG CMIA (Abbot Diagnostics, Wiesbaden, Germany) during the transplantation work-up, and recorded as reactive/positive (+) or non-reactive/negative (-). The results were combined to define the CMV serostatus as D-/R-, D+/R-, D+/R+ and D-/R+. All patients received universally leucodepleted blood products.

As per institutional protocol, quantitative CMV PCR was measured on plasma in IU/mL by Roche COBAS Ampliprep/COBAS TaqMan CMV test (Roche Molecular Systems, Branchburg, NJ, USA), (CMV DNA analytical sensitivity 56 IU/mL, linear range 137-9x10^6 IU/mL) twice weekly from day +1 until at least day +100 or immunosuppression was weaned. CMV reactivation was defined as the detection of any CMV DNA in the peripheral blood, while clinically significant CMV infection (cs-CMVi) was defined as CMV viral load of >1000 IU/mL. CMV disease was diagnosed by tissue biopsy of involved organs or detection of CMV DNA by PCR in bronchoalveolar lavage, and classified by ECIL 7 criteria.[9,25] Cs-CMVi was treated with pre-emptive therapy of renally adjusted induction dose ganciclovir (5mg/kg BID), or valganciclovir (900mg BID), or if neutropenic, foscarnet (90mg/kg BD) until CMV viral load <1000 IU/mL or a minimum of two weeks, then renally adjusted maintenance dose (half induction dose) until CMV clearance or a minimum of two weeks.

Spontaneous clearance

Clearance of viremia was defined as two consecutive negative CMV PCR values. Spontaneous clearance was defined as clearance of viremia, in the absence of CMV antiviral therapy. Any patient with CMV viral load >1000 IU/mL was pre-emptively treated as previously described, and patients were excluded from the spontaneous clearance analysis who were initiated on pre-emptive therapy prior to a CMV viral load >1000 IU/mL with high dose valacyclovir/acyclovir (>1g TID), valganciclovir/ganciclovir, foscarnet or letermovir. All patients with a documented positive CMV PCR result were included for the purposes of this analysis.

Statistical Analyses

All end-points were assessed from the day of transplant. Cumulative incidence functions were used for CMV reactivation, cs-CMVi, CMV disease and GVHD, with death as a competing risk. Relapse and NRM were competing risks for each other. The association of these outcomes with pre-transplant factors were analyzed using Fine-Gray competing risk regression.[31] OS and disease free survival (DFS) were calculated using Kaplan-Meier analysis and groups compared with the Log-rank test, with the impact of pre-transplant variables assessed using Cox regression. Post-transplant variables (cs-CMVi, aGVHD) were analysed as time-dependent covariates within regression models. Variables with a p value of <0.10 on univariate analysis were included into multivariate models, with a p value of ≤ 0.05 considered significant. The proportional hazards assumption was checked using Schoenfeld residuals. Given the collinearity between donor source and GVHD prophylaxis (as all URD were administered either ATG or corticosteroids), we constructed two separate multivariate regression analyses: "Model 1" with donor source, and "Model 2" which separated GVHD prophyalxis into ATG and corticosteroid "Ruutu" cohorts. Patients who proceeded to a second alloHCT were analysed from the date of the first transplant, with variables arising from the second transplant not included to avoid duplication. Statistical analyses were performed using R statistical software version 3.5.2 (R Core Development Team, Vienna, Austria) PlotsOfDifference[32] and EZR.[33]

RESULTS

CMV reactivation and Clinically Significant CMV Infection

There were 160 consecutive alloHCT recipients during the study period. One patient who received letermovir was excluded from the analysis, with 159 patients meeting inclusion criteria (Table 1). The cumulative incidence of CMV reactivation was 56% (90/159), with 35% (56/159) progressing to cs-CMVi and requiring pre-emptive therapy. Cs-CMVi was most common with D-/R+ serostatus at 63% (25/40), compared to 38% (28/74) of D+/R+, 18% (2/11) of D+/R- and 0% in D-/R- ($P<0.01$, excluding D-/R-), (Figure 1). URD recipients had a higher rate of cs-CMVi (40%, 37/93) compared to MRDs (26%, 16/62), ($P<0.01$). On univariate analysis, cs-CMVi was associated with URD, HLA mismatched donor, D-/R+ serostatus, and use of ATG or corticosteroids as GVHD prophylaxis. On multivariate analysis, only URD and D-/R+ serostatus remained significant (Table 2). Cs-CMVi rates in R+ differed according to donor CMV serostatus and donor source: 76% D-/R+URD [n=25] vs 43% D+/R+URD [n=40], 43% D-/R+MRD [n=14] and 29% D+/R+MRD [n=31] ($P<0.01$) (Figure 1).

Spontaneous CMV Clearance and Timing of Reactivation

Eighty four alloHCTs reactivated CMV and were assessed for spontaneous clearance (58 D+/R+, 30 D-/R+ and 3 D+/R-). Seven patients were excluded from the analysis due to off-protocol early initiation of anti-

CMV therapy prior to a CMV viral load >1000 IU/mL (3 high dose valacyclovir [1 D+/R+URD, 1 D+/R+MRD, 1 D-/R+URD], 4 ganciclovir/valganciclovir [3 D+/R+URD, 1 D+/R+MRD]). A total of 4409 quantitative CMV viral loads were evaluated, of which 784 results tested positive. Rates of spontaneous clearance are shown in Figure 3 (see also Supplementary Table S1). 33% (28/84) spontaneously cleared CMV prior to reaching the pre-emptive threshold of 1000 IU/mL. Spontaneous clearance occurred only at lower viral loads; for patients with a viral load of 56-137 IU/mL, 26% (22) spontaneously cleared CMV, 6% (5) spontaneously cleared CMV after a viral load of 138-250 IU/mL, and only 1 patient cleared CMV if the viral load increased to >250 IU/mL. Fifty-six patients did not spontaneously clear their CMV viral load before levels increased to >1000 IU/mL and pre-emptive therapy was initiated. MRD were more likely to spontaneously clear CMV than URD at a viral loads of 56-137 IU/mL (MRD 47% vs URD 19%, P=0.03), D+/R+ were more likely to spontaneously clear CMV than D-/R+ with CMV viral loads of 56-137 IU/mL (D+/R+ 45% vs D-/R+ 13%, P<0.01). MRD were also more likely to spontaneously clear CMV than URD at any viral load >250 IU/mL (MRD 44% vs URD 23%, P=0.04), and likewise D+/R+ were more likely to spontaneously clear CMV than D-/R+ at any viral load >250 IU/mL (D+/R+ 43% vs D-/R+ 13%, P<0.01).

The median time to first CMV reactivation (>56 IU/mL) was 26 days (95% CI, 24 – 32; range, 6 - 179), CMV viral load >250 IU/mL was 39 days (95% CI, 33 – 40; range, 11 - 175) and >1000 IU/mL was 41 days (95% CI, 39 – 46; range 11 - 195), (See Figure 4 and Supplementary Table S2). URD reactivated CMV more quickly, with median time to viral load of >250 IU/mL at 36 days (95% CI, 31 – 39) vs 43 days (95% CI, 39 – 50) in MRD (P<0.01). In patients who did not spontaneously clear CMV (n=54), the median time between first CMV reactivation (>56 IU/mL) and viral load >250 IU/mL was 13 days (95% CI, 11 – 14), while the time from first viral load >250 IU/mL to reach vial loads >1000 IU/mL was 4 days (95% CI, 3 – 4), (See Supplementary Table S3).

Twenty-seven percent of patients (15/56) who required pre-emptive therapy had a second reactivation and 5% (3/56) a third reactivation event. The CMV serostatus and donor source of these reactivating patients were: D-/R+URD (9/25, 36%; 5 MMURD, 4 MURD), D-/R+MRD (3/14, 21%), D+/R+URD (5/40, 13%; 2 MMURD, 3 MURD), D+/R+MRD (1/31, 3.2%).

CMV Disease

The cumulative incidence of CMV disease was 6.3% and 7.2% at 1 and 3 years respectively; with the cumulative incidence of proven and probable CMV disease being 3.1% and 4.0% respectively. Eleven patients met criteria for proven or probable CMV disease (Table 1; 7 colon, 1 esophageal, 1 pneumonitis, 1 retinitis, 1 cardiac). Proven or probable CMV disease was more common in D-/R+URD (6/25, 24%; 4 D-/R+MMURD, 2 D-/R+MURD) compared to D+/R+MRD (3/31, 7.6%), with 1 D+/R-URD (MURD), and 1 D+/R+ haploidentical recipient, and no disease in D-/R+MRD or D+/R+URD.

Impact of CMV on Survival

NRM was 10.1% (95% CI 6.0 – 15.3) at day 100 and 16.4% (95% CI 11.1 – 22.6) at 1 year. OS was 87.4% (95% CI 81.2 – 91.7) by day 100, 71.0% (95% CI 63.2 – 77.4) at 1 year and 59.4% at 3 years (95% CI 51.6 – 68.4). DFS was 81.8% (95% CI 74.8 – 86.9) by day 100, 60.8% (95% CI 52.2 – 67.4) at 1 year and 48.3% (95% CI 39.6 – 56.6) at 3 years. Univariate and mulitvariate analysis of pre- and post-tranplant variables for NRM, OS and DFS are shown in Tables 3-5. On multivariate analysis of pre-transplant variables, D-/R+ serostatus was associated with significantly increased NRM (HR 2.92, 95% CI 1.07 – 7.99, P=0.03), together with URD and RIC (Table 3). On multivariate analysis using time-dependent variables RIC and cs-CMVi were the only predictors of NRM (HR 2.98, 95% CI 1.35 – 6.57, P <0.01). When analysing CMV seropositive patients, D-/R+URD [n=25] recipients demonstrated the highest NRM, 32% compared to 20% D+/R+URD [n=40], 5.1% D-/R+MRD [n=14] and 9.7% D+/R+MRD [n=31], (P=0.04) (Figure 2).

On univariate analysis of factors impacting of DFS, age, URD and ATG use were considered significant. On multivariate analysis of pre-transplant factors only, age, URD and ATG had a significant impact on DFS. When incorporating time dependent variables in the multivariate model there was no impact of cs-CMVi or aGVHD on DFS.

On univariate analysis of factors impacting on OS, age, URD, ATG or corticosteroid use as GVHD prophylaxis, development of aGVHD and cs-CMVi were significant. On multivariate analysis using pre-transplant variables only, age, URD, and ATG use were considered significant predictors of OS. When incorporating time dependent variables in the model, only URD and ATG use were significantly associated with OS. D-/R+URD recipients demonstrated a worse 1-year OS at 52% compared to 73% D+/R+URD, 93% D-/R+MRD and 74% D+/R+MRD (P=0.04). Only one death was attributable to CMV disease, secondary to CMV pneumonitis.

Additionally, any CMV reactivation compared to no CMV reactivation, and CMV reactivation with a spontaneous clearance compared no CMV reactivation, was not associated with an increase in NRM (HR 1.27, 95% CI 0.63 – 2.59, P=0.50; and HR 0.21, 95%CI 0.02 – 1.96, P=0.17, respectively); or OS (HR 1.16, 95% CI 0.69 – 1.96, P=0.57; and HR 0.90, 95% CI 0.42 – 1.91, P=0.79, respectively).

Incidence of aGVHD

The incidence of aGVHD was 23.3%, with no difference based on CMV serostatus (D-/R- 24% vs D+/R- 36% vs D-/R+ 30% vs D+/R+ 18%, P=0.30), donor type (URD 24% vs MRD 24%, P=0.67), HLA mismatch (MURD 24% vs MMURD 24%, P=0.99) or conditioning intensity (RIC 26% vs MAC 13%, P=0.17), (See Supplementary Table S4). The development of grade II-IV aGVHD had no significant impact on cs-CMVi, NRM, OS or DFS (Tables 2-5).

DISCUSSION

This study has identified pre- and post-transplant factors which could be used to predict the groups at highest risk of post alloHCT CMV complications and more effectively tailor CMV pre-emptive and prophylactic strategies. Our results suggest that when using a standardised pre-emptive approach, CMV serostatus and donor type are significant predictors of CMV reactivation, cs-CMVi and NRM, with D-/R+ URD being the group at highest risk of CMV related complications in our analysis. We have noted that cs-CMVi was associated with an increased risk of NRM in our patient population. Moreover, spontaneous CMV clearance was more common in MRD versus URD and D+/R+ compared to D-/R+, and spontaneous clearance was uncommon once viral loads exceeded 250 IU/mL.

Previous reports have identified a number of factors predicting risk of CMV reactivation including CMV serostatus, donor type, use of T-cell depletion or post-transplant cyclophosphamide, GVHD and steroid use.[15,21,34-39] Our results match these earlier findings.[36,38,40] Cs-CMVi was associated with an increase in NRM in our cohort. Previous studies have suggested an increase in NRM associated with CMV reactivation.[1,3,9] It is possible that the use of pre-emptive antiviral therapy may account for some of this impact on NRM since we could show no mortality impact of spontaneously resolving CMV viremia. Importantly, our work has identified D-/R+ URD as a particularly high risk cs-CMVi group, with higher NRM; identifying a group most likely to benefit from CMV prophylaxis. Reserving CMV prophylaxis to D-/R+ URD, and maintaining a pre-emptive approach to lower risk groups may limit cost and toxicity while optimising outcome.

Historically, comparing CMV viral loads between laboratories has been problematic because of variations in assay performance and reporting standards. However, with the recent International standardization in the method of CMV quantitation (World Health Organization standard International Unit or IU/mL), viral load values between different laboratories can now be compared.[3,41] This advancement has allowed comparison between studies as well as allow the results of these studies to be applied by alloHCT facilities internationally. Therefore, international standardization of pre-emptive therapy using the most appropriate CMV viral threshold to start anti-CMV treatment is now possible.

A recent study assessing spontaneous clearance of CMV by Camargo *et al.* demonstrated that delaying initiation of therapy until a CMV viral load is ≥350 IU/mL was associated with protracted CMV viremia and that unresolved viremia by treatment day 35 was associated with increased NRM.[15] This suggests that delaying treatment may result in longer treatment duration with an associated increase in toxicity, cost and mortality. A limitation of the study was that pre-emptive therapy was initiated at varying viral loads at the discretion of the treating clinician, without a clearly defined threshold. In contrast, our study used a strict threshold for the initiation of pre-emptive therapy together with twice weekly CMV viral

load until at least day 100. This approach permitted an assessment of the utility of pre-emptive therapy when initiated at a fixed threshold of 1000 IU/ml but also allowed us to determine lower thresholds associated with progression of CMV viral load, as well as rates of spontaneous clearance at thresholds <250 IU/ml. Few patients spontaneously cleared CMV who had a CMV viral load >250 IU/mL without pre-emptive therapy, demonstrating that there is little benefit in delaying pre-emptive therapy once viral loads exceed >250 IU/mL. Our data also suggests that thresholds for spontaneous clearance varied with donor source/serostatus so that thresholds for pre-emptive therapy could be initiated at >250 IU/mL for D+ MRD, and for any D-/R+ or URD with a CMV viral load >137 IU/mL; avoiding unnecessary delays in pre-emptive therapy which may translate in shorter treatment duration, lower cost and less toxicity. We found that the median time between first CMV reactivation (>56 IU/mL) and a viral load >250 IU/mL was 13 days, whereas the time from viral load of >250 IU/mL to >1000 IU/mL was 4 days. This suggests that clinicians can cautiously "watch-and-wait" for spontaneous clearance with viral loads <250 IU/mL, while pre-emptive treatment could be initiated once viral loads exceed 250 IU/ml. Furthermore, our results demonstrate that the timing of reactivation differs according to donor source and CMV serostatus, with URD reactivating CMV and developing a cs-CMVi earlier than MRD, and D+/R- demonstrating cs-CMVi as late onset infections (after day 60) compared to R+ patients; which is consistent with the literature,[35] however our D+/R- population was too small (n=11) to draw any significant conclusions for this group.

In our centre, all URD received either corticosteroids (pre 2015, n=23) or ATG (post 2015, n=73) as GVHD prophylaxis. While we could not demonstrate an impact of corticosteroids or ATG on NRM, ATG use had a significant impact on OS and DFS on multivariate analysis. Previous reports have suggested a significant impact on mortality when ATG has been used in recipients with D-/R+ serostatus. This effect was reported by Kalra *et al.* in a large retrospective cohort study in ATG treated alloHCT which demonstrated that D-/R+ patients had a substantially lower survival than D+/R+ patients (41% versus 59% at 5 years; P<0.001).[42] A possible explanation for this increased risk has been described by Schmidt-Hieber *et al.* who demonstrated that *in vivo* T cell depletion with ATG influenced both the CMV infection risk and T cell reconstitution kinetics post alloHCT.[43] The authors demonstrated that by day +30 post HSCT, CMV-specific CD8+ T cells were detected in 57% of D+ patients, but in none of the D-/R+ patients. The combination of T cell depletion and inability to generate a CMV specific T cell response post alloHCT may contribute to the increased risk of cs-CMVi and subsequent mortality shown in the D-/R+ URD subgroup.

In addition, a recent study by Talaya *et al.* in a T-cell replete patient population with minimal exposure to ATG (17%), found that episodes of CMV that resolved spontaneously below a pre-emptive threshold of >1500 IU/mL had no impact on mortality.[44] Another study by Solano *et al.*,[45] in which there was no T cell depletion or ATG use, showed similar findings.

Interestingly, we could not show a significant impact of ATG on rates of cs-CMVi. While this may have been a function of sample size, it is notable that we could not demonstrate a difference in rates of grade II-IV aGVHD according to donor source in our population. This effect has been previously described.[46] Given that the development of grade II-IV aGVHD would normally be associated with a high risk of CMV reactivation,[38] it may be that the lower than expected rates of aGVHD in URD recipients who received routine corticosteroids or ATG as GVHD prophylaxis in our cohort, explains why we could not demonstrate an impact of ATG or aGVHD on cs-CMVi.

Limitations of the current study include the relatively small sample size from a single centre. However, our cohort is relatively homogenous and was managed according to a standard protocol which included twice weekly CMV monitoring, standardized initiation of pre-emptive therapy, and with near 100% follow-up. Since we aimed to assess which pre-transplant factors were most relevant for CMV reactivation, we limited our assessment of post-transplant factors predicting CMV reactivation to the development of aGVHD. Additional studies which assess the impact of other post-transplant factors such as neutropenia, post-alloHCT maintenance chemotherapy, or concomitant infections may also identify other high risk cohorts where CMV prophylaxis could be justified. Primary GVHD prophylaxis in this cohort was predominantly cyclosporin with post-transplant methotrexate and therefore our results cannot be extrapolated to other cohorts using alternative GVHD prophylaxis such as post-transplant cyclophosphamide, mycophenolate or mTOR inhibitors such as sirolimus.

In conclusion, our study indicates D-/R+ URD recipients represent a high risk cohort for cs-CMVi and CMV related mortality, and therefore are potential candidates for CMV prophylaxis. Additionally, we have demonstrated that spontaneous clearance of CMV above the viral load of 250 IU/mL is uncommon, suggesting that there is little benefit in delaying pre-emptive therapy for recipients of alloHCT whose CMV viral load exceeds this threshold.

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TABLE LEGEND

Table 1.

Baseline pre-transplant characteristics of all patients with cumulative incidence of any CMV detection, clinically significant CMV infection (cs-CMVi) and CMV disease

Table 2.

Univariate and multivariate analysis of pre and post-transplant factors associated with clinically significant CMV infection (cs-CMVi, >1000 IU/mL)

Table 3.

Univariate and multivariate analysis of pre and post-transplant factors associated with non-relapse mortality (NRM)

Table 4.

Univariate and multivariate analysis of pre and post-transplant factors associated with overall survival (OS)

Table 5.

Univariate and multivariate analysis of pre and post-transplant factors associated with disease free survival (DFS)

FIGURE LEGEND

Figure 1.

Clinically significant CMV infection (csCMVi) of (A.) all alloHCT by serostatus (n=159, P<0.01 excluding D-/R-) and (B.) of CMV+ alloHCT by serostatus and donor type (n=125, P<0.01)

Figure 2.

CMV R+ alloHCTs by serostatus and donor type (A.) Non-relapse mortality (n=125, P=0.02) and, (B.) Overall survival (n=125, P=0.01)

Figure 3.

Rate of spontaneous clearance of CMV by (A.) viral load (IU/mL) for all patients to reactivate CMV (n=84), and (B.) by viral load in D+/R+ vs D-/R+ and matched related donor (MRD) vs unrelated donor (URD)

Figure 4.

Difference in time of first CMV viral load by thresholds >56 IU/mL, >250 IU/mL and >1000 IU/mL (cs-CMVi) in all patients (n=84), unrelated donor (URD, n=48) and matched related donor (MRD, n=32)

Figure 4 Footnote:

The summary of the data is shown as a violinplot reflecting the data distribution and a vertical line indicating the median. A horizontal bar indicates for each median the 95% confidence interval determined by bootstrapping. The plot on the right shows the effect size, relative to "All patients first level >56".

Table 1. Baseline pre-transplant characteristics of all patients with cumulative incidence of any CMV detection, clinically significant CMV infection (cs-CMVi) and CMV disease

Characteristic	N (%)	Any CMV detected	cs-CMVi (>1000/mL)	CMV Disease
Total	159	56%	35%	6.3%
Median age [Range]	56.5 [20 – 71]			
Age 20-50	51 (32%)	53%	29%	12%
Age 50-60	46 (29%)	50%	30%	4.3%
Age 60-71	62 (39%)	63%	42%	3.2%
Gender				
Female	72 (45%)	71%	40%	4.6%
Male	87 (55%)	44%	30%	8.3%
Primary Disease				
Acute Myeloid Leukemia (AML)/Myelodysplastic syndrome (MDS)	87 (55%)	61%	36%	4.6%
Acute Lymphobstic Leukemia (ALL)	12 (7.5%)	67%	50%	17%
Lymphoma	36 (23%)	47%	31%	8.3%
Other	24 (15%)	46%	29%	4.2%
Donor type				
Matched related donor (MRD)	62 (39%)	53%	26%	1.6%
Mismatched related donor (MMRD)	1 (0.6%)	100%	100%	100%
Unrelated donor (URD)	93 (59%)	56%	40%	7.5%
Haploidentical donor	3 (1.9%)	67%	67%	33%
HLA mismatch URD				
No (matched unrelated donor, MURD)	55 (35%)	49%	34%	5.1%
Yes (mismatched unrelated donor, MMURD)	38 (25%)	71%	50%	16%
CMV Serology				
D-/R-	34 (21%)	0.0%	0.0%	0%
D+/R-	11 (6.9%)	27%	18%	9.1%
D+/R+	74 (47%)	77%	38%	4.1%
D-/R+	40 (25%)	73%	63%	15%
Conditioning Intensity				
Myeloablative conditioning (MAC)	30 (19%)	56.7%	20%	6.7%
Reduced intensity conditioning (RIC)	129 (81%)	55.8%	38%	6.2%
GVHD prophylaxis				
Antithymocyte globulin (ATG)	73 (46%)	52.1%	36%	9.6%
Corticosteroids	23 (15%)	69.6%	48%	4.3%
Neither	63 (40%)	55.6%	29%	3.2%

Table 2. Univariate and multivariate analysis of pre and post-transplant factors associated with clinically significant CMV infection (cs-CMVi, >1000 IU/mL)

Clinically significant CMV infection (cs-CMVi, >1000 IU/mL)						
	Univariate			Multivariate		
	HR	95% CI	p value	HR	95% CI	p value
Pre-transplant risk factors						
Age (by year)	1.01	0.99 – 1.04	0.29			
HCT-CI Score						
1-2	1.33	0.69 – 2.53	0.394			
≥3	1.00	0.32 – 3.09	0.998			
Disease[†]	0.98	0.57 – 1.69	0.94			
DRI Score						
Intermediate	1.59	0.38 – 6.59	0.52			
High/Very High	2.09	0.48 – 9.03	0.32			
Unrelated donor (URD)[‡]	**2.13**	**1.21 – 3.76**	**<0.01**	**2.06**	**1.10 – 3.86**	**0.02**
HLA mismatch	**1.85**	**1.01 – 3.38**	**0.05**	1.08	0.55 – 2.12	0.82
Reduced intensity conditioning (RIC)	2.08	0.88 – 4.93	0.10	2.24	0.94 – 5.33	0.07
CMV serology[§]						
D+/R+	2.51	0.66 – 9.46	0.18	2.31	0.63 – 8.45	0.21
D-/R+	**5.28**	**1.42 – 19.63**	**0.01**	**4.89**	**1.35 – 17.68**	**0.02**
GVHD prophylaxis						
Antithymocyte globulin (ATG)	**2.04**	**1.11 – 3.75**	**0.02**	1.95	0.97 – 3.90	0.06
Corticosteroids	**2.06**	**1.00 – 4.24**	**0.05**	1.70	0.79 – 3.66	0.17
Post-transplant events (time dependent variables)						
aGVHD (≥ Grade II)	1.19	0.59 – 2.40	0.62			

Excludes D-/R- group which had no csCMVi. Excludes haploidentical (3) and mismatched related donor (MMRD) (1) patient/s due to low numbers. Donor source and GVHD prophylaxis assessed in separate multivariate analysis models due to high degree of collinearity, all other variables unaffected between models. Abbreviations: aGVHD, acute graft versus host disease; HCT-CI, Hematopoietic cell transplant comorbidity index; DRI, Disease risk index.

[†]All other diseases compared to AML/MDS as baseline group

[‡]Compared to matched related donor (MRD) recipients as baseline group.

[§]Compared to D+/R- serostatus as baseline group

Table 3. Univariate and multivariate analysis of pre and post-transplant factors associated with non-relapse mortality

Non-relapse mortality (NRM)									
	Univariate			Multivariate (pre-transplant variables only)			Multivariate (with time dependent variables)		
	HR	95% CI	p value	HR	95% CI	p value	HR	95% CI	p value
Pre-transplant risk factors									
Age (by year)	1.02	0.99 – 1.04	0.15						
HCT-CI									
1-2	1.39	0.61- 3.14	0.427						
≥3	0.93	0.234 – 3.74	0.924						
Disease†	1.10	0.56 – 2.16	0.78						
DRI									
Intermediate	0.81	0.183 – 3.57	0.780						
High/Very High	1.92	0.42 – 8.76	0.398						
Unrelated donor (URD)‡	2.09	0.99 – 4.40	**0.05**	**2.27**	**1.10 – 4.69**	**0.03**	1.97	0.96 – 4.05	0.07
HLA mismatch	1.77	0.85 – 4.40	0.13						
Reduced intensity conditioning (RIC)	**7.94**	**1.10 – 57.6**	**0.04**	**8.97**	**1.28 – 62.91**	**0.03**	6.84	0.98 – 48.00	**0.05**
CMV serology¶									
D+/R-	0.63	0.08 – 4.86	0.65	0.79	0.10 – 6.50	0.83	0.64	0.08 – 5.14	0.67
D+/R+	1.39	0.50 – 3.86	0.53	1.53	0.55 – 4.27	0.42	0.99	0.36 – 2.73	0.99
D-/R+	**2.70**	**0.99 – 7.39**	**0.05**	**2.92**	**1.07 – 7.99**	**0.04**	1.40	0.48 – 4.09	0.53
GVHD prophylaxis									
Antithymocyte globulin (ATG)	1.92	0.88 – 4.17	0.14						
Corticosteroid	2.10	0.78 – 5.57	0.14						
Post-transplant events (time dependent variables)									
aGVHD (≥ Grade II)	**2.31**	**1.06 – 5.02**	**0.04**				1.96	0.88 – 4.33	0.10
Cs-CMVi (>1000 IU/mL)	**4.15**	**1.94 – 8.87**	**<0.01**				**2.98**	**1.35 – 6.57**	**<0.01**

Excludes haploidentical (3) and mismatched related donor (MMRD) (1) patient/s due to low numbers. Donor source and GVHD prophylaxis assessed in separate multivariate analysis models due to high degree of collinearity, all other variables unaffected between models.

Abbreviations: aGVHD, Acute graft versus host disease; HCT-CI, Hematopoietic cell transplant comorbidity index; DRI, Disease risk index.

†All other diseases compared to AML/MDS as baseline group

‡Compared to MRD as baseline group.

¶Compared to D-/R- serostatus as baseline group

Table 4. Univariate and multivariate analysis of pre and post-transplant factors associated with Overall Survival

Overall Survival (OS)									
	Univariate			Multivariate (pre-transplant variables only)			Multivariate (with time dependent variables)		
	HR	95% CI	p value	HR	95% CI	p value	HR	95% CI	p value
Pre-transplant risk factors									
Age (by year)	**1.02**	**1.00 – 1.04**	**0.07**	**1.02**	**1.00 – 1.05**	**<0.05**	1.02	1.00 – 1.05	0.08
HCT-CI									
1-2	0.93	0.47 – 1.83	0.83						
≥3	0.6	0.20 – 2.07	0.50						
Disease[†]	1.00	0.61 – 1.67	0.97						
DRI									
Intermediate	1.01	0.31 – 3.29	0.99	0.90	0.27 – 2.99	0.86	0.88	0.27 – 2.91	0.83
High/Very High	2.85	0.85 – 9.49	0.089	2.19	0.64 – 7.43	0.21	1.97	0.58 – 6.74	0.28
(Unrelated donor) URD[‡]	**2.25**	**1.28 – 3.94**	**<0.01**	**2.02**	**1.11 – 3.66**	**0.02**	**1.99**	**1.10 – 3.60**	**0.02**
HLA mismatch	1.45	0.81 – 2.61	0.21						
Reduced intensity conditioning (RIC)	1.70	0.81 – 3.57	0.16						
CMV serology[¶]									
D+/R-	0.22	0.03 – 1.69	0.14						
D+/R+	1.16	0.59 – 2.28	0.66						
D-/R+	1.45	0.71 – 2.98	0.31						
GVHD prophylaxis									
Antithymocyte globulin (ATG)	**2.06**	**1.14 – 3.73**	**0.02**	**1.89**	**1.02 – 3.53**	**0.04**	**1.96**	**1.06 – 3.62**	**0.03**
Corticosteroid	**2.23**	**1.08 – 4.62**	**0.03**	2.10	0.97 – 4.53	0.06	1.85	0.84 – 4.03	0.12
Post-transplant events (time dependent variables)									
aGVHD (≥ Grade II)	**1.84**	**1.03 – 3.30**	**0.04**				1.69	0.93 – 3.05	0.08
Cs-CMVi (>1000 IU/mL)	1.70	1.00 – 2.89	**0.05**				1.30	0.75 – 2.27	0.35

Excludes haploidentical (3) and mismatched related donor (MMRD) (1) patient/s due to lack of significant numbers. Donor source and GVHD prophylaxis assessed in separate multivariate analysis models due to high degree of collinearity, all other variables unaffected between models. Abbreviations: aGVHD, Acute graft versus host disease; HCT-CI, Hematopoietic cell transplant comorbidity index; DRI, Disease risk index.

[†]All other diseases compared to AML/MDS as baseline group

[‡]Compared to MRD as baseline group.

¶Compared to D-/R- serostatus as baseline group

Table 5. Univariate and multivariate analysis of pre and post-transplant factors associated with disease free survival (DFS)

Disease Free Survival (DFS)									
	Univariate			Multivariate (pre-transplant variables only)			Multivariate (with time dependent variables)		
	HR	95% CI	p value	HR	95% CI	p value	HR	95% CI	p value
Pre-transplant risk factors									
Age (by year)	**1.02**	**1.00 - 1.04**	**0.07**	**1.02**	**1.00 - 1.04**	**<0.05**	1.02	1.00 – 1.04	0.06
HCT-CI									
1-2	0.88	0.47 - 1.63	0.68						
≥3	0.49	0.15 - 1.53	0.22						
Disease[†]	1.10	0.70 - 1.71	0.68						
DRI									
Intermediate	1.01	0.36 - 2.82	0.98	0.94	0.33 - 2.65	0.91	0.93	0.33 – 2.63	0.89
High/Very High	2.72	0.95 - 7.77	0.06	2.20	0.76 - 6.39	0.15	2.14	0.73 – 6.21	0.16
Unrelated donor (URD)[‡]	**2.01**	**1.23 - 3.28**	**0.01**	**1.77**	**1.05 - 2.97**	**0.03**	**1.77**	**1.05 – 2.97**	**0.03**
HLA mismatch	1.18	0.69 - 2.03	0.55						
Reduced intensity conditioning (RIC)	1.45	0.77 - 2.75	0.25						
CMV serology[¶]									
D+/R-	0.72	0.24 - 2.17	0.56						
D+/R+	1.17	0.65 - 2.10	0.60						
D-/R+	1.17	0.61 - 2.25	0.63						
GVHD prophylaxis									
Antithymocyte globulin (ATG)	**2.05**	**1.22 - 3.45**	**<0.01**	**1.88**	**1.09 - 3.25**	**0.02**	**1.93**	**1.12 – 3.33**	**0.02**
Corticosteroid	1.86	0.95 - 3.63	0.07	1.62	0.79 - 3.32	0.19	1.54	0.74 – 3.15	0.24
Post-transplant events (time dependent variables)									
aGVHD (≥ Grade II)	1.44	0.83 - 2.49	0.19				1.3	0.77 – 2.31	0.38
Cs-CMVi (>1000 IU/mL)	1.29	0.78 - 2.08	0.30				0.99	0.59 – 1.62	0.94

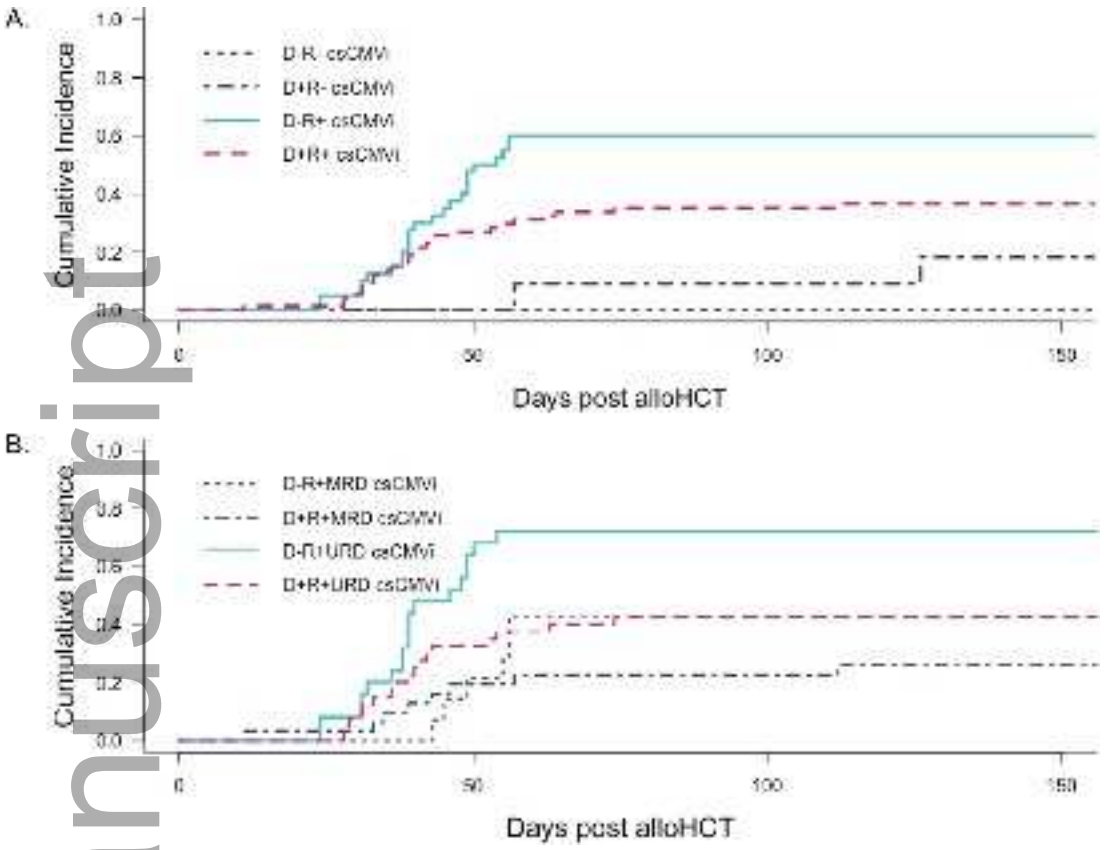
Excludes haploidentical (3) and mismatched related donor (MMRD) (1) patient/s due to low numbers. Donor source and GVHD prophylaxis assessed in separate multivariate analysis models due to high degree of collinearity, all other variables unaffected between models.

Abbreviations: aGVHD, Acute graft versus host disease; HCT-CI, Hematopoietic cell transplant comorbidity index; DRI, Disease risk index.

[†]All other diseases compared to AML/MDS as baseline group

[‡]Compared to MRD as baseline group.

¶Compared to D-/R- serostatus as baseline group



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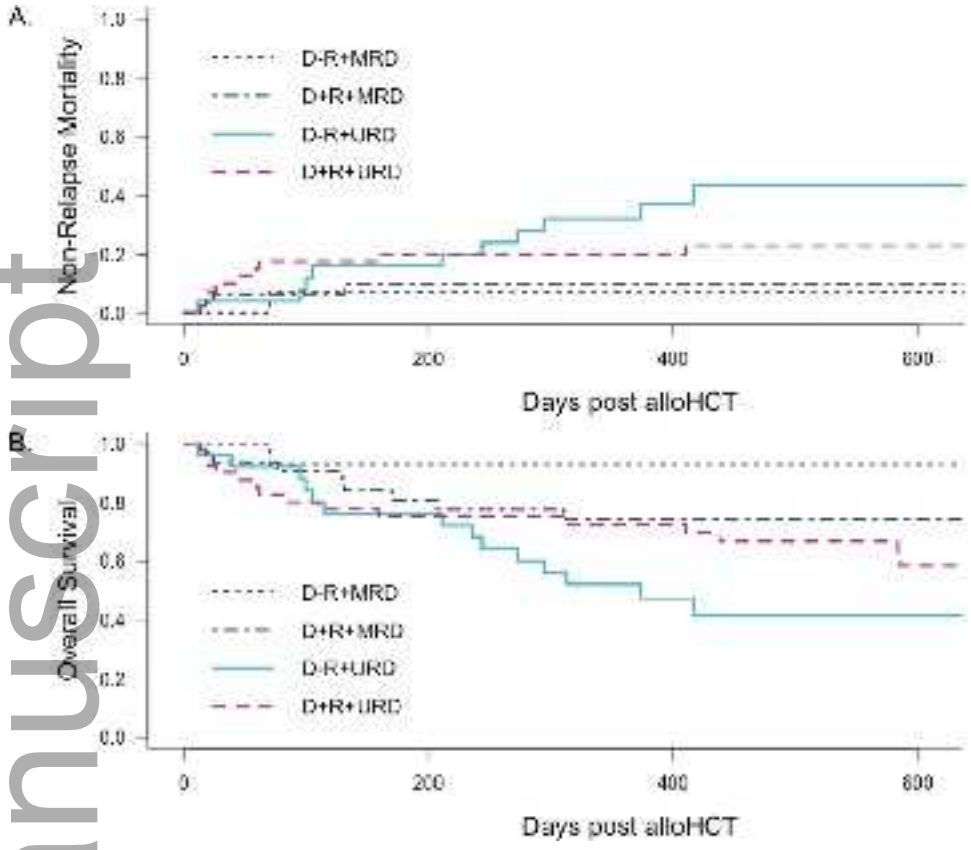

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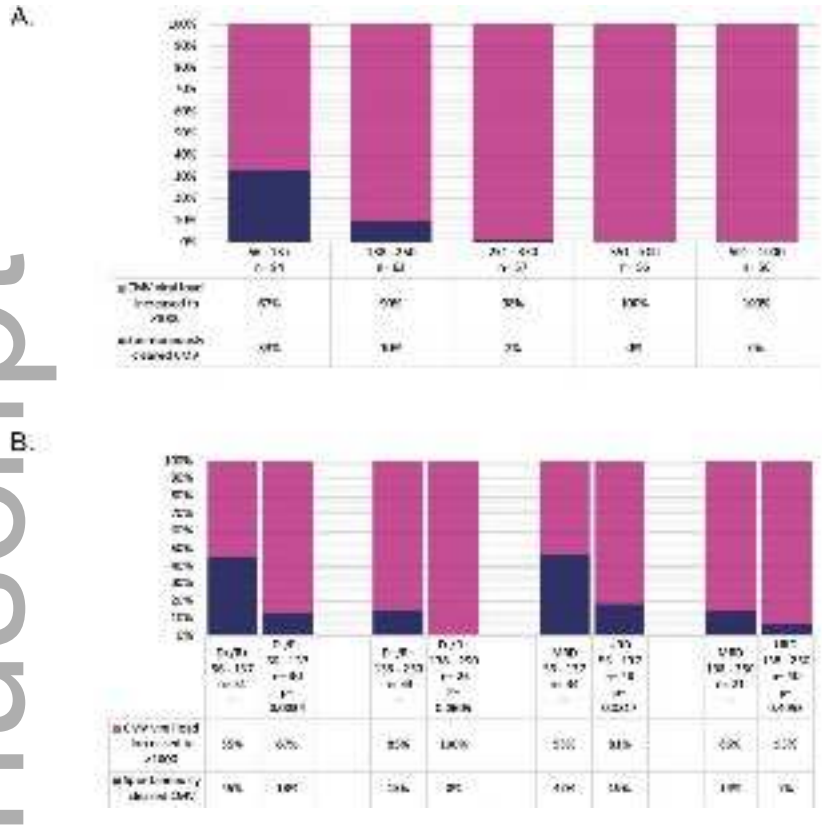

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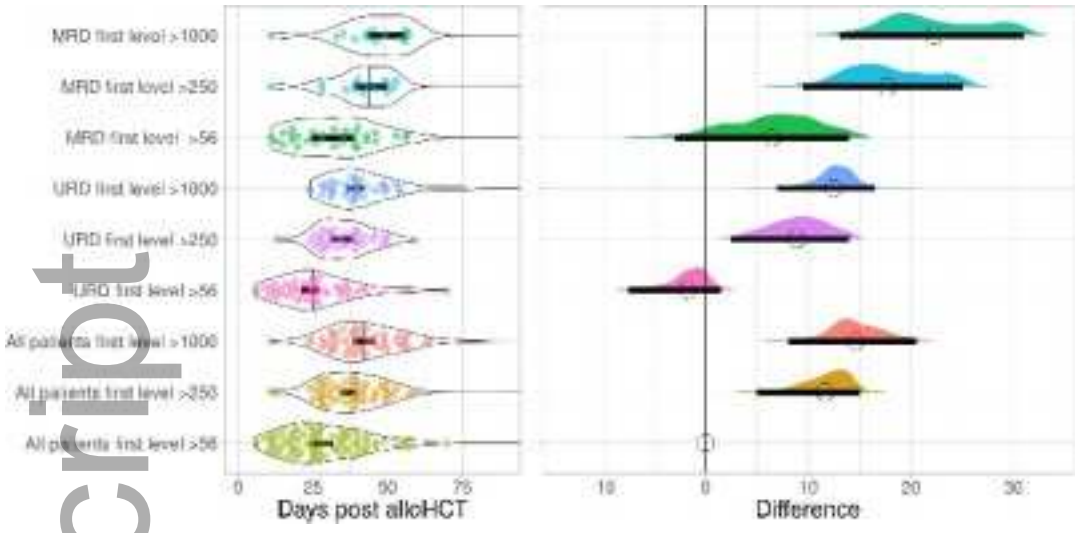

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