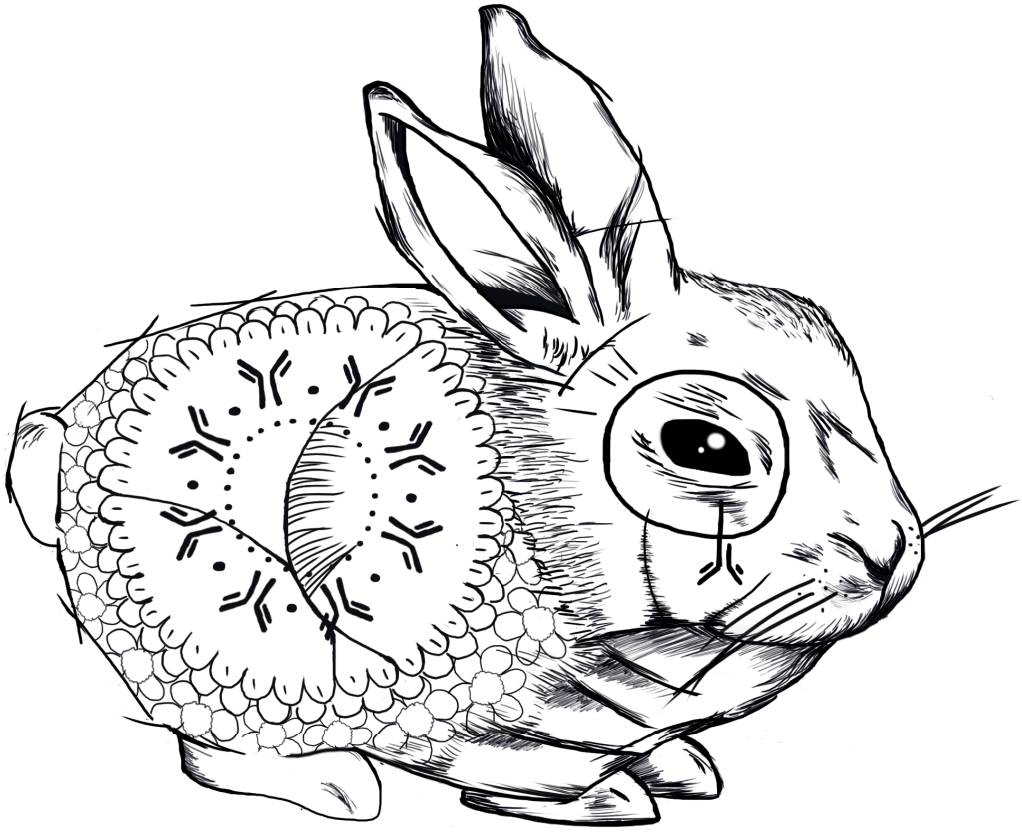

Chapter 10

Viral Reactivations and Associated Outcomes in Immune Reconstitution after Pediatric Hematopoietic Cell Transplantation

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ABSTRACT

Background

Viral reactivations (VR) following hematopoietic cell transplantation (HCT) contribute to significant morbidity and mortality. Timely immune reconstitution (IR) is suggested to prevent VR. We studied the relation between IR (as a continuous over-time-predictor) and VR (as time-varying-predictor), and the relation between VR and other clinical outcomes.

Patients and Methods

In this retrospective analysis, all patients receiving a first HCT between January-2004 and September-2014 were included. IR (CD3/CD4/CD8 T-cells, NK- and B-cells) was measured bi-weekly until 12 weeks, and monthly thereafter. Main outcomes of interest were VR of adenovirus (AdV), Epstein-Barr-virus (EBV), human-herpesvirus 6 (HHV6), cytomegalovirus (CMV), and BK-virus, screened weekly. Clinical outcomes included overall-survival (OS), event-free-survival, non-relapse-mortality (NRM), and graft-versus-host-disease (GvHD). Cox-proportional-hazard- and Fine-Gray-competing-risk-models were used.

Results

273 patients (0.1-22.7 years; median follow-up 58 months) were included. CD4-reconstitution predicted reactivation of AdV (HR 0.995; p=0.022), EBV (HR 0.994, p=0.029), and HHV6 (HR 0.991, p=0.012), but not CMV (p=0.31) and BK (p=0.27). Duration of AdV-reactivation was shorter with timely CD4-reconstitution, defined as $\geq 50*10^6$ cells/L within 100-days. AdV-reactivation predicted lower OS (HR 2.17, p=0.0039) and higher NRM (HR 2.96, p=0.0008). Concomitant CD4-reconstitution abolished this negative effect of AdV-reactivation: OS (p=0.67) and NRM (p=0.64). EBV- and HHV6-reactivations were predictors for occurrence of GvHD, while CMV- and BK-reactivations did not predict clinical outcomes.

Conclusion

These results stress the importance of timely CD4-reconstitution. Strategies to improve CD4-reconstitution may improve HCT-outcomes, including survival, and reduce the need for toxic anti-viral therapies.

INTRODUCTION

Allogeneic hematopoietic cell transplantation (HCT) is a potentially curative therapy for various hematological malignancies and benign disorders in children. The success of HCT is hampered by relapse of malignancy and transplantation related mortality due to HCT-associated complications, which include opportunistic infections (e.g. viral reactivations; VR) and graft-versus-host disease (GvHD). The majority of these limitations are due to absent or delayed T-cell reconstitution.[1,2]

Early CD4+ T-cell reconstitution in particular is suggested to be important for better survival chances after HCT.[2–7] Delayed immune reconstitution (IR) after HCT was previously found to be a predictor for VR,[7] which may subsequently contribute to acute GvHD (aGvHD), graft failure (GF), and increased mortality.[8] Early and improved prediction of HCT-related complications provides the opportunity for earlier initiation of pre-emptive treatment, which subsequently could improve survival chances.

Although VR and IR are related and are both associated with clinical outcome,[7–10] their joint time-dependent relation as predictors for clinical outcome remains to be studied. Most studies investigating the role of IR-markers as a predictor for VR only include very limited measurements at relative late time-points after HCT. As most VRs occur early after HCT, this is not optimal. Early IR-markers assessed as a continuous value over time may provide better predictors, which can also be used in clinical practice for decision-making. The identification of early IR-related predictors may guide us in the initiation of anti-viral therapies or targeted anti-viral cellular therapies.

We aimed to assess the relation between IR and VR and other clinical outcomes. To achieve this we did a large retrospective cohort analysis in which clinical outcomes, such as survival, GvHD, and GF, were related to VR. In this analysis, VR was uniquely evaluated as a time-varying predictor. Additionally, this is the first study that related various immune reconstitution markers as continuous over-time variables to viral reactivations. This enabled us to determine the effect of immune reconstitution on VR-associated clinical outcomes.

PATIENTS AND METHODS

Study design and patients

In this analysis we included pediatric patients receiving an allogeneic HCT, between January 2004 and September 2014 at the University Medical Centre in Utrecht, The Netherlands. All consecutive patients undergoing their first transplantation were included. The minimum

follow-up for surviving patients was 6 months, and data were collected and registered prospectively. Patients were enrolled and data were collected only after written informed consent in accordance with the Helsinki Declaration. The study was approved by the local ethical committee (trial numbers 05-143 and 11-063k).

Procedures

Conditioning regimens were applied according to (inter-)national protocols. For myeloablative busulfan-containing protocols (administered intravenously) therapeutic drug monitoring was used to aim for an area under the curve (AUC) of 75-95 mg*h/L. Anti-thymocyte globulin (ATG; Thymoglobulin) was given at a dose of 10 mg/kg starting 5 days before HCT from 2004 to 2010. From 2010 onwards, patients weighing over 40 kg received a lower dose of ATG (7.5 mg/kg). A 50% dose reduction was given when pre-conditioning lymphocyte counts were less than $300*10^6$ T-cells/L. Those receiving a cord blood transplant from 2009 on received ATG starting 9 days before HCT. Starting 2013, patients receiving a cord blood transplant for malignant indications did not receive ATG. Patients received gut decontamination and infection prophylaxis according to local protocols. GvHD-prophylaxis consisted of cyclosporin A (CsA; targeted at trough levels of 150-250 µg/L for all patients), combined with either prednisolone 1 mg/kg (cord blood) or methotrexate 10 mg/m^2 (on day +1, +3, and +6; unrelated donor). CsA was continued for at least 3 months or 6 months after HCT in patients with benign and malignant indication, respectively. Prednisolone was tapered after 28 days in benign disorders, and after engraftment in malignancies. Cord blood recipients were treated with filgrastim from day +7 after HCT until neutrophils were above 2000 cells/µL. Patients were treated in high-efficiency, particle-free, air-filtered, positive-pressure isolation rooms.

Cytomegalovirus (CMV) and Epstein-Barr virus (EBV) serostatus was assessed in all patients and donors prior to HCT. Following transplantation, all patients underwent weekly PCR viral screening for Human Herpesvirus 6 (HHV6), EBV, Adenovirus (AdV), CMV, and BK-virus irrespective of serostatus and clinical conditions. Viral reactivation, in which no distinction was made between reactivation or primo infection, was defined as having a viral load of >1000 copies/mL. From 2012 onwards, EBV and CMV were reported in IU/mL, for which the threshold was set at 5000 and 500 IU/mL, respectively, according the conversion factor in the same viral laboratory. Patients showing an EBV-, CMV- or AdV-reactivation received anti-viral treatment. Patients with HHV6-reactivation were treated only in high clinical suspicion of HHV6-associated disease (e.g. encephalitis, bone marrow suppression). Rituximab was used for treating EBV-reactivation; CMV was treated with either ganciclovir or foscarnet, HHV6 with foscarnet, and AdV-reactivation with cidofovir. Patients with BK-virus associated hemorrhagic cystitis received hyperhydration (3 L/m^2/day) as supportive care. Treatment for viral reactivations was stopped when viral load was

undetectable in two subsequent samples, or in case of severe toxicity after acquiring low viral loads (<1000 copies/mL).

After reaching a leukocyte count of at least $0.3*10^9$/L, absolute numbers of lymphocyte subsets, including overall T-cells (CD3+), T helper-cells (CD3+CD4+CD8-), cytotoxic T-cells (CD3+CD8+CD4-), B-cells (CD19+), and NK-cells (CD3-CD16+CD56+), were measured by flow cytometry at least every other week up to twelve weeks post-HCT and monthly thereafter up to six months post-HCT on EDTA-treated whole blood using TruCOUNT technology (BD Biosciences, Erembodegem, Belgium).

Outcomes

Main outcome of interest: Viral reactivations, of AdV, EBV, HHV6, CMV, and BK, defined as viraemia with >1000 copies/mL.

Other outcomes of interest: Overall survival (OS) was defined as time from transplantation to death or last follow-up. Event-free survival (EFS) was defined as time from HCT to last contact whereby graft-failure, relapse of disease, or death were regarded as events. All surviving patients were censored at date of last contact. Non-relapse mortality (NRM) was defined as death due to a cause other than relapse of malignancy. Relapse related mortality (RRM) was defined as death due to relapse of a malignancy. Acute- and chronic GvHD (aGvHD, cGvHD) were classified according to the Glucksberg[11] and Shulman[12] criteria, respectively. Graft failure was defined as non-engraftment (autologous reconstitution) or graft rejection (secondary loss of donor chimerism). In case of non-engraftment, the assessment date was +60 days after HCT. Interstitial pulmonary syndrome (IPS) and bronchitis obliterans (BO) were defined according to international accepted criteria as previously described.[13]

Statistical analysis

Duration of the follow-up was defined as the time from HCT to the last assessment for surviving patients or death. Actual cell counts for CD3+, CD4+ and CD8+ T-cells, as well as NK- and B-cells, considered as continuous values over time, were evaluated as predictors for viral reactivation. For the clinical endpoints, viral reactivations of AdV, EBV, HHV6, CMV, and BK were evaluated as predictors. Additionally, reconstitution of CD4+ T-cells was assessed as a co-predictor for clinical outcome. This was chosen in line with previous findings.[2,10,14,15] CD4+ T-cell reconstitution (CD4+ IR) was defined as reaching $\geq 50*10^6$ CD3+CD4+ cells/L in 2 consecutive measurements within 100 days post-HCT, and considered as time-varying covariate, in accordance with previous publications.[14] In multivariate analysis, predictors considered for outcome were patient-related variables (age at transplant, gender, CMV and EBV serostatus), disease (malignant/primary immune deficiencies; PID/bone marrow failure/benign non-PID), donor factors (HLA disparity, CMV and EBV serostatus), and conditioning regimen (myeloablative or reduced intensity conditioning).

As immune suppression was similar for all patients during the first 3 months after HCT, this was not considered as a multivariate predictor. Variables associated with a p-value <0.05 by univariate analysis were selected for testing in a multivariate analysis. Probabilities of events were calculated using the Kaplan-Meier estimate; the two-sided log-rank test was used for univariate comparisons. Time-dependent outcomes were analyzed using Cox proportional hazard models. For the endpoints NRM, RRM, aGvHD, cGvHD, and GF, Fine-Gray competing risk regressions were used. Here, competing events were RRM, NRM, death not due to aGvHD or cGvHD, and death not due to GF, respectively. Statistical analyses were performed using R 3.0.1 using the packages survival and cmprsk.

Role of the Funding Source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. RA and JJB had full access to all the data in the study and RA, CdK, and JJB had final responsibility for the decision to submit for publication.

RESULTS

A total of 273 patients were included with a median age of 8.4 years (range 0.1-22.7). Patient characteristics are summarized in Table 1. Busulfan combined with fludarabine and ATG (Thymoglobulin) was the most frequently used conditioning regimen. Cord blood (52%, n=142) and bone marrow (45%, n=123) were the most frequently used cell sources. Median time to follow-up was 58 months (range 0.2-130). Cumulative incidence of viral reactivations was 27%, 18%, 15%, 13% and 11% for HHV6, CMV, AdV, BK and EBV, respectively (Fig S2).

We only identified CD4+ IR as a predictor for VR. No associations between CD3+, CD8+ T-cells, NK-, or B-cell IR and VR were found (Supplemental Table S1). In AdV, the chance on reactivation is reduced 5% with every increase of 10/µl CD4+ T-cell counts (hazard ratio [HR] 0.995, 95% confidence interval [CI]: 0.99-0.999, p=0.022; Table 2). CD4+ IR similarly predicted EBV (HR 0.994, 95% CI: 0.988-0.999, p=0.029), and HHV6 (HR 0.991, 95% CI: 0.985-0.998, p=0.012). No relation with CMV-reactivation (p=0.31) and BK-reactivation (p=0.27) was identified.

Interestingly, patients with successful early CD4+ IR had shorter treatment duration of AdV (Figure 1). The median treatment duration of was only 14 days for patients with timely CD4+ IR, compared to 47.5 days for patients without recovery of CD4+ IR (p=0.011). Early CD4+ IR did not influence the duration of CMV, EBV and HHV6 (Supplemental Figure S3). Timely CD4+ IR, however, did also not affect duration of treatment for CMV (p=0.29; Supplemental Figure S3a).

	All patients
Number of patients (n)	273
Male sex (%)	59
Age at transplant (years)	8.4 (0.1-22.7)
Recipient/Donor EBV Serostatus	
-/-	19
+/-	60
-/+	3
+/+	18
Recipient/Donor CMV Serostatus	
-/-	37
+/-	48
-/+	3
+/+	12
Diagnosis (%)	
Malignancy	53
Primary immune deficiency	12
Bone marrow failure	16
Benign non-PID	18
Stem cell source (%)	
Bone Marrow	45
Cord Blood	52
Peripheral blood stem cells	3
Conditioning regimen (%)	
Busulfan based	83
TBI based	17
Patients receiving serotherapy (%)	71
Follow-up (months)	58 (0.2-130)

Table 1. patient characteristics

We next investigated the effects of viral reactivations on clinical outcome parameters. In this analysis, patients were considered as having a VR only from the day of reaching >1000 viral copies/mL in plasma, giving a robust estimation of the hazard associated with the different VR's (Figure S1). Here, we identified AdV, EBV, and HHV6 as VR-predictors for various clinical outcomes (Table 3). Reactivation of AdV was a predictor for lower OS (HR 2.17, 95% CI 1.28-3.68, p=0.0039; Table 3, Figure 2A) and EFS (HR 1.77, 95% CI 1.05-2.99, p=0.032; Table 3, Figure 2C) chances compared to patients not experiencing an AdV-reactivation (HR 2.17, 95% CI 1.28-3.68, p=0.0039; Table 3, Figure 2A). We did not find any VR associated with RRM (Table S4). NRM was associated with both AdV- (HR 2.95, 95% CI 1.57-5.57, p=0.0008; Table 3, Figure 2E) and EBV-reactivations (HR 2.03, 95% CI 1.01-4.11, p=0.049, respectively; Figure S4A).

Endpoint	HR	95% confidence interval	p-value	Significance level
CMV-reactivation				
CD4+ T-cell counts (continuous over time)	0.998	0.993 – 1.002	0.312	
HHV6-reactivation				
CD4+ T-cell counts (continuous over time)	0.991	0.985 – 0.998	0.012	*
BK-virus-reactivation				
CD4+ T-cell counts (continuous over time)	0.996	0.99 – 1.003	0.271	
AdV-reactivation				
CD4+ T-cell counts (continuous over time)	0.995	0.99 – 0.999	0.022	*
EBV-reactivation				
CD4+ T-cell counts (continuous over time)	0.994	0.988 – 0.999	0.029	*

Table 2. Multivariate analysis of CD4+ IR versus VR. IR was considered as a continuous time-varying predictor. VR was defined as having a viral load >1000 copies/mL in plasma. * Indicates p<0·05. HR are presented as the hazard ratio for each point increase in CD4+ T-cell counts.

As early CD4+ IR predicted both the incidence and duration of VR, we subsequently investigated whether CD4+ IR influenced the negative effect of VR on clinical outcome. Here, we found that OS was similar in patients with AdV-reactivation and concurrent CD4+ IR compared to those patients without an AdV-reactivation (68±3% vs 66±12%, p= 0.67; Figure 2B). Patients with AdV-reactivation without CD4+ IR performed considerably worse (OS of 32±10; p=0.0045 in MV analysis for CD4+ IR; Table 3). For EFS and NRM, we found comparable results: patients with AdV-reactivation without CD4+ IR had lower EFS (63% and 62% versus 32% for AdV-, AdV+/IR+, and AdV+/IR-, respectively; p=0.0021 in MV analysis for CD4+ IR; Table 3) and higher NRM (18% and 28% versus 54% for AdV-, AdV+/IR+, and AdV+/IR-, respectively; p=0.0005 in MV analysis for CD4+ IR; Table 3, Figure S4B) compared to those with CD4+ IR in presence or absence of AdV.

While AdV was associated with all survival-related parameters, HHV6 and EBV were predictors for the occurrence of GvHD. HHV6 was found to be a predictor for a higher chance on aGvHD grade 2-4 (HR 3.47, 95% CI 2.11-5.7, p<0.0001; Table 3, Figure S5A). Diagnosis was an additional multivariate predictor with bone marrow failure and primary immune deficiencies having the lowest chance on grade 2-4 aGvHD (Table S3). For grade 3-4 aGvHD, both HHV6 (HR 2.74, 95% CI 1.22-6.15, p=0.015; Table 3, Figure S5B) and EBV (HR 4.8, 95% CI 1.31-17.62, p=0.018; Table 3, Figure S5C) were predictors, with age

being an additional predictor (Table S3). Extensive chronic GvHD (cGvHD) probability was predicted by EBV-reactivation (HR 3.61, 95% CI 1.13-11.53, p=0.03; Table 3, Figure S5D). Receiving cord blood as cell source was an additional predictor for lower chance on cGvHD (Table S3). For the outcomes GF, lung injury, IPS, and BO, we did not identify any VR-predictors.

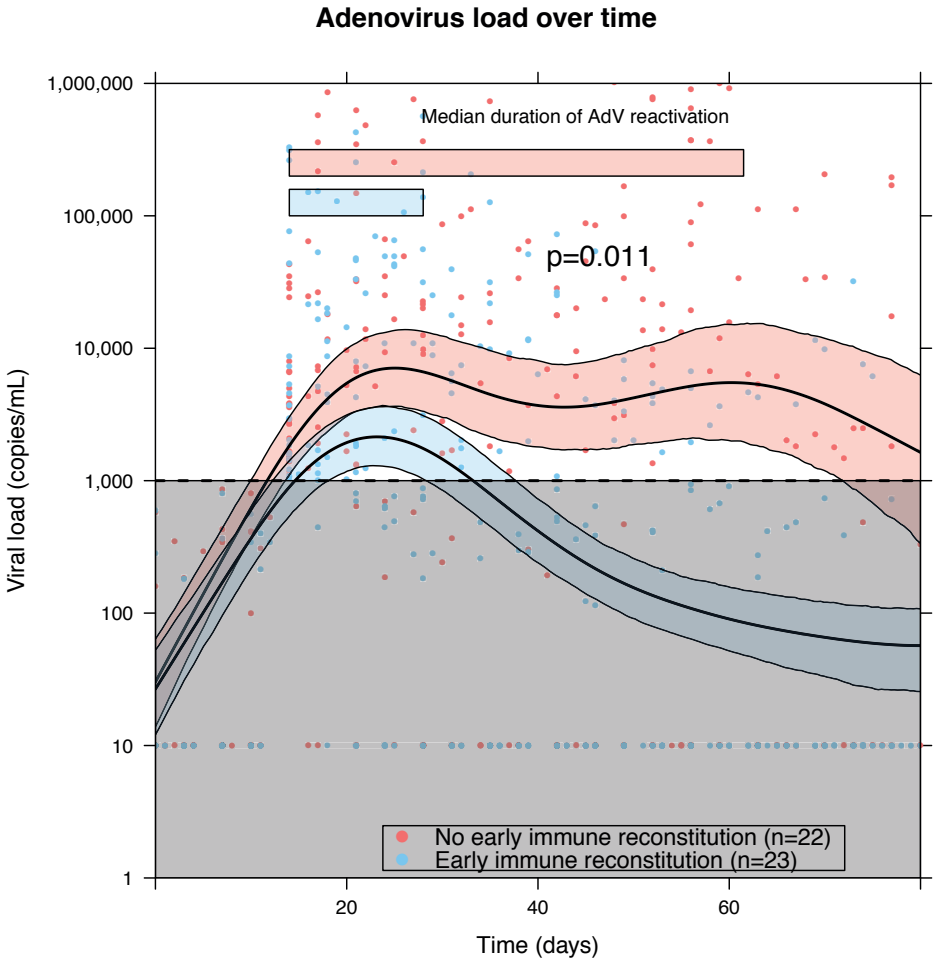

Figure 1. Viral load in patients with AdV reactivation, according to early reconstitution of CD4+ T- cells. Spline regression analysis (5 degrees of freedom) of AdV load (bold solid lines with 95% CI as shaded area) over time (normalized to 14 days before reaching a viral load of 1000) in patients with AdV-reactivation, with (blue area/dot/bar) or without (red are/dot/bar) early CD4+ T-cell reconstitution, showing longer AdV-reactivation and higher AdV loads in those without early reconstitution. Median duration of AdV-treatment was 14 days for patients with early immune reconstitution, and 47.5 days for the no early immune reconstitution group. Dots: individual raw AdV load. Bars: median duration of AdV treatment. Grey background: clinically insignificant viral load.

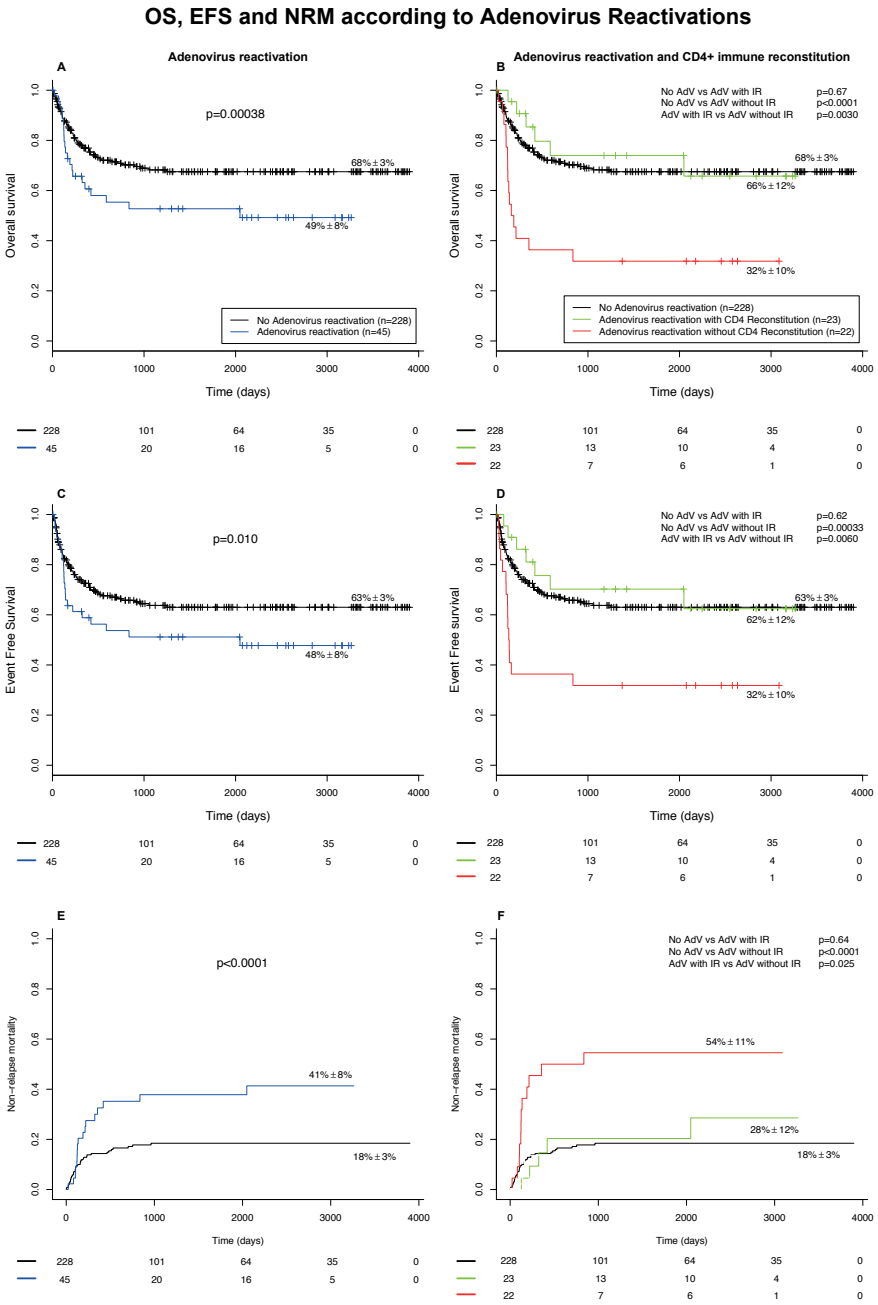

Figure 2. Clinical outcome according to viral reactivation and immune reconstitution. Plots on the left show the incidence of A: overall survival (OS), C: Event Free Survival (EFS), and E: Non-relapse Mortality (NRM), in patients without AdV-reactivation (black lines) versus patients with AdV- reactivation (blue lines). Viral reactivation was defined as having a viral load >1000 copies/mL. Plots on the right show the effect of AdV and CD4+ T-cell reconstitution on the incidence of B: OS, D: EFS, and F: NRM, in patients without AdV-reactivation (black lines) versus patients with AdV-reactivation; subdivided into patients having CD4+ T-cell reconstitution (green) versus patients not having CD4+ T-cell reconstitution (red)

	Univariate	Multivariate			
Variable	**p-value**	**HR**	**95% CI**	**p-value**	
Overall Survival					
AdV-reactivation >1000 copies/mL	0.00038	2.173	(1.282-3.684)	0.0039	**
Early CD4+ T-cell reconstitution	0.002	0.52	(0.332-0.816)	0.0045	**
Event Free Survival					
AdV-reactivation >1000 copies/mL	0.0101	1.771	(1.05-2.988)	0.032	*
Early CD4+ T-cell reconstitution	0.00079	0.515	(0.337-0.786)	0.0021	**
Non-Relapse Mortality					
AdV-reactivation >1000 copies/mL	<0.0001	2.955	(1.567-5.571)	0.0008	***
EBV-reactivation >1000 copies/mL	0.0013	2.028	(1.001-4.109)	0.049	*
Early CD4+ T-cell reconstitution	0.00017	0.344	(0.189-0.629)	0.0005	***
Grade 2-4 acute GvHD					
HHV6-reactivation >1000 copies/mL	<0.0001	3.472	(2.113-5.704)	<0.0001	****
Grade 3-4 acute GvHD					
EBV-reactivation >1000 copies/mL	0.0085	4.799	(1.307-17.616)	0.018	*
HHV6-reactivation >1000 copies/mL	0.014	2.74	(1.22-6.155)	0.015	*
Extensive chronic GvHD					
EBV-reactivation >1000 copies/mL	0.0298	3.611	(1.131-11.534)	0.03	*

Table 3. Multivariate analysis of VR and CD4+ IR versus outcome. VR and IR were considered as time varying predictors. VR was defined as having a viral load >1000 copies/mL in plasma. * Indicates $p<0.05$, ** $p<0.01$, *** $p<0.001$, and **** $p<0.0001$.

DISCUSSION

To our knowledge this is the first study to investigate the relations between various immune reconstitution markers, occurrence and duration of VR, and clinical outcome in a large pediatric cohort. Here, we uniquely considered all predictors in a time-dependent manner, making the findings more robust. With the limitations of a retrospective cohort study taken into account, our data show that from all evaluated immunological markers, timely CD4+ T-cell reconstitution predicted reactivations of AdV, EBV, and HHV6 best. Furthermore, AdV predicted lower OS and EFS, AdV and EBV predicted higher NRM, while EBV- and HHV6-reactivation predicted GvHD. Importantly, early CD4+ IR completely abolished the adverse effect of VR on survival parameters. Moreover, CD4+ IR did not influence reactivations of CMV and BK, nor were these VR's predictive for clinical outcome.

Our finding that absent of timely CD4+ IR predicts VR is in line with previous findings.[2–7,14] A previously suggested relation between CD8+ T-cell recovery and VR could not be identified[16], nor could a correlation between T-cell recovery and CMV-reactivation be confirmed.[7,15] These discrepancies may be due to the fact that we considered IR as a

continuous, time-dependent variable as early as 2 weeks after HCT, rather than binary and time-independent at certain time-points as done in most previous analyses. By taking time-dependency into account, a more precise insight into the predictive value of the various the various immune-markers will be obtained. Nonetheless, in the implemented models, an effect of viraemia on CD4+ T-cell counts is not accounted for, although most patients do not show an increase in CD4+ count after VR.

Considering VR as predictors for clinical outcome, our findings are in partly line with other studies showing that AdV-reactivation is associated with lower survival,[17] EBV is associated with lower survival and GvHD,[18] and HHV6-reactivation is associated with a higher risk for aGvHD in myeloablative HCT recipients.[19,20] Some conflicting data exist as well, since others did find a relation between HHV6 and higher mortality rates,[20,21] or between HHV6- or EBV-reactivation and aGvHD.[22,23] These discrepancies may most likely be explained by differences in treatment protocols, age, or VR definition. Again here, considering the time-dependency of the variables may also have had a significant influence the found associations.

With respect to CMV-reactivation in relation to clinical outcome, we did not find any association, while CMV has previously been associated with the occurrence of aGvHD and lower survival.[8,15] This may be due to the pre-emptive treatment in our cohort controlling the negative effects that are associated with CMV. However, most available data on the impact of CMV-reactivations is acquired through retrospective multi-center registry studies, where CMV monitoring may differ between the centers: e.g. when CMV-load is only measured when suspicion of CMV-disease, there may be an under-reporting of actual CMV reactivations. While a significant effect of CMV on survival was shown in large registry studies, the absolute effect size, and thereby the clinical relevance, was relatively small (difference of 1.8% in OS and ~5% in NRM in studies by EBMT,[24] and CIBMTR,[25] respectively. Also, BK-reactivation was found not to predict any of the clinical outcome parameters assessed in this study, however we did not consider hemorrhagic cystitis as an outcome measure. Regardless of the fact that BK associated cystitis can be a very painful and a long lasting complication requiring prolonged hospitalization,[26] according to our findings it does not affect survival or any other clinical outcome analyzed after HCT.

Data from this study imply that anti-viral therapies to increase survival chances in case of VR might not be needed in patients with timely CD4+ IR, especially taking toxicities of anti-viral drugs (e.g. nephrotoxicity, bone marrow suppression) into account. Thus, anti-viral treatment may be delayed and possibly omitted in cases with sufficient CD4+ T-cell recovery at the time of reactivation. Early monitoring of CD4+ IR can be an important tool

to identify patients at risk. The effect and safety of omitting anti-viral therapy in patients with CD4+ IR should, however, be carefully evaluated first.

Although this association between absence of CD4+ IR and lower survival was described previously,[1,3,5,6] it has never been shown before that CD4+ IR impacts VR-associated mortality. Therefore, an important strategy to prevent mortality associated with VR would be to target for a predictable and certain early CD4+ IR after HCT. As recent findings by our group suggest that high exposure of ATG after HCT significantly delays CD4+ IR,[14] individualized ATG dosing regimens, aiming at optimal exposure, may fasten CD4+ IR, and subsequently enhance CD4+ T-cell predictability. Other strategies that might stimulate CD4+ IR are currently being tested in clinical settings; e.g. sex hormone inhibitors,[27] low-dose interleukin-2 treatment,[28] keratinocyte growth factor,[29] and Thymosin alpha 1 treatment.[30] The effects of these treatment options on early CD4+ T-cell recovery, however, remain to be explored.

In conclusion, the results obtained by this study stress the importance of having timely CD4+ IR, and provide insight in morbidities and mortality associated with developing a viral reactivation. Strategies to better predict CD4+ IR are of utmost importance to improve survival chances after HCT. These novel strategies should, preferably, be tested in the context of harmonized clinical trial design and standardized immuno-monitoring to better compare different strategies, as recently reviewed.[31] Moreover, identifying patients with early CD4+ IR, and thus at lower risk for viral reactivation-related morbidity and mortality, may limit the need for toxic anti-viral drugs. On the other hand, the identification of at risk patients with a delayed CD4+ IR provides the opportunity to pre-emptively intervene with anti-viral (cell) therapies. Altogether, finding strategies that lead to a better predictable CD4+ T-cell reconstitution and subsequently the prevention of viral reactivation may lead to lower morbidity and better survival chances for HCT patients.

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