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Immune Reconstitution and Infection Patterns after Early Alemtuzumab and Reduced Intensity Transplantation for Nonmalignant Disorders in Pediatric Patients

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A B S T R A C T

Hematopoietic stem cell transplantation (HSCT) is a therapeutic option for many nonmalignant disorders (NMD) and is curative or prevents disease progression. Reduced-intensity conditioning (RIC) in HSCT for NMD may reduce regimen-related acute toxicities and late complications. Myeloablation is often replaced by immune suppression in RIC regimens to support donor engraftment. The pace of immune reconstitution after immune suppression by RIC regimens is influenced by agents used, donor source, and graft-versus-host disease prophylaxis/treatment. In a multicenter trial (NCT 00920972) of HSCT for NMD, a RIC regimen consisting of alemtuzumab, fludarabine, and melphalan was substituted for myeloablation. Alemtuzumab was administered early (days −21 to −19) to mitigate major lymphodepletion of the incoming graft and the risk of graft rejection. Immune reconstitution and infectious complications were prospectively monitored for 1-year post-HSCT. Seventy-one patients met inclusion criteria for this report and received marrow or peripheral blood stem cell transplants. Immune reconstitution and infections are reported for related donor (RD) and unrelated donor (URD) transplants at 3 time-points (100 days, 6 months, and 1 year post-HSCT). Natural killer cell recovery was rapid, and numbers normalized in both cohorts by day +100. Mean CD3, CD4, and CD8 T-lymphocyte numbers normalized by 6 months after RD HSCT and by 1 year in the URD group. CD4 and CD8 T-lymphocyte counts were significantly higher in patients who received RD HSCT at 6 months and at 1 year, respectively, post-HSCT compared with patients who received URD HSCT. The pace of CD19 B-cell recovery was markedly different between RD and URD cohorts. Mean B-cell numbers were normal by day 100 after RD HSCT but took 1 year post-HSCT to normalize in the URD cohort. Despite these differences in immune reconstitution, the timing and nature of infections did not differ between the groups, presumably because of comparable T-lymphocyte recovery. Immune reconstitution occurred at a faster pace than in prior reports using RIC with T-cell depletion. The incidence of infections was similar for both cohorts and occurred most frequently in the first 100 days post-HSCT. Viral and fungal infections occurred at a lower incidence in this cohort, with “early” alemtuzumab

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compared with regimens administering serotherapy in the peritransplantation period. Patients were susceptible to bacterial infections primarily in the first 100 days irrespective of donor source and had no increase in mortality from the same. The overall mortality rate from infections was 1.4% at 1 year. Close monitoring and prophylaxis against bacterial infections in the first 100 days post-HSCT is necessary but is followed by robust immune reconstitution, especially in the T-cell compartment.

INTRODUCTION

Hematopoietic stem cell transplantation (HSCT) is a curative therapeutic option for nonmalignant disorders (NMD), because donor engraftment can provide hematopoietic, immune, or cellular-enzyme-based control of a variety of disorders [1–4]. Traditionally, NMD HSCT has used myeloablative conditioning (MAC) to facilitate eradication of host stem cells and donor cell engraftment, which is especially important in overcoming rejection in an immune-competent host [5]. However, MAC conditioning predominately includes irradiation or busulfan-based regimens and is associated with significant toxicities such as bone marrow aplasia, mucositis, malnutrition, end-organ dysfunction, and gonadal failure [1].

Reduced-intensity conditioning (RIC) regimens that are immunosuppressive rather than myeloablative can support successful donor engraftment and may have fewer regimen-related toxicities and late complications [6–11]. However, the use of RIC has its own inherent limitations, including delayed immune reconstitution, infections, and graft rejection [8,12-14]. A multicenter prospective trial (NCT 00920972) evaluated a novel immunosuppressive RIC regimen for transplantation of pediatric patients with NMD. Host immunosuppression included "early" alemtuzumab, followed by fludarabine, and melphalan. This regimen demonstrated successful donor engraftment and favorable event-free survival of 83% to 93% as previously published [15–17].

Because alemutuzumab has a long half-life of approximately 2 weeks, and administration in proximity to the stem cell infusion could result in graft T-cell depletion, rejection, and delayed immune reconstitution, the timing of alemtuzumab administration was placed early in the regimen (day −21) [8,18-21]. We hypothesized that this strategy, combined with peritransplant fludarabine and melphalan, would allow early clearance of alemtuzumab, efficient depletion of the host immune system optimizing engraftment, and robust immune reconstitution post-HSCT. Immune reconstitution, crucial for a successful post-transplantation recovery, is also influenced by donor source and graft-versus-host disease (GVHD) prophylaxis/treatment. We report on the pace of immune reconstitution after RIC and the incidence of infection in the first year post-HSCT following this RIC regimen in pediatric patients enrolled on NCT 00920972.

METHODS

Patients

Patients ≤21 years of age with NMD were enrolled on this prospective trial between December 2001 and August 2011 at participating centers. Eligibility included availability of marrow or peripheral blood stem cells from an HLA-matched or one-antigen-mismatched related donor (RD) or unrelated donor (URD). Patients met standard organ function requirements pretransplantation and were required to be free of active invasive infections for 4 weeks before conditioning.

Patients were included in this report if they had successful engraftment by day 100 and had immune reconstitution data available for at least 2 of the 3 time points sought (days 100, 6 months, 1 year).

Treatment Regimen

All patients received alemtuzumab, fludarabine, and melphalan [15-17]. Alemtuzumab was administered intravenously for 3 consecutive days between days −21 and −19, after a test dose of 3 mg. Patients <10 kg received 33 mg of alemtuzumab, and all other patients received 48 mg as previously described [22]. Fludarabine (30 mg/m^2/day; 1 mg/kg/day in patients <10 kg) was administered on days −8 to −4. Melphalan (140 mg/m^2; 4.7 mg/kg in patients <10 kg) was administered on day −3.

Donor Selection

All patients received HSCT from a matched or 1-antigen-mismatched RD or URD [15,22]. When high-resolution typing was used, donors were considered eligible if matched at 7 or 8 HLA loci (-A, -B, -C, and -DRB1) with the recipient. Three patients who received matched URD HSCT were typed by low resolution and matched at -A, -B, and -DRB1 HLA loci are included in this analysis. Bone marrow was the preferred stem cell source, but peripheral blood stem cells were used when marrow was not available. Subanalysis based on HLA-match or stem cell source was not performed due to small sample size.

Supportive Care

Patients received GVHD prophylaxis as previously described including tacrolimus or cyclosporine, short-course methotrexate (on days +1, 3, and 6) in all, and short-course prednisone (until day +28 and tapered over a month in URD HSCT recipients) [15-17,22]. Systemic immunosuppression was tapered after 6 months in the absence of GVHD.

All patients received ciprofloxacin and itraconazole or alternate broad spectrum antifungal prophylaxis until day +100. Granulocyte-colony stimulating factor (5 ug/kg/day) was given starting on day +7 until the absolute neutrophil count was ≥500 cells/μL for 3 consecutive days. Cytomegalovirus (CMV), Epstein-Barr virus (EBV), and adenovirus replication were monitored weekly and treated pre-emptively.

Study Definitions and Evaluation Criteria

Neutrophil engraftment was defined as the first of 3 days that the absolute neutrophil count was ≥500 cells/μL after the nadir. Lymphocyte subpopulations (CD3, CD4, CD8, CD19, and CD16/56) were measured in peripheral blood at day 100, 6 months, and 1-year post-HSCT. The lower limit of normal was defined as 1000 cells/μL (CD3), 500 cells/μL (CD4), 300 cells/μL (CD8), 200 cells/ μL (CD19), and 40 cells/μL (CD16/56) [23]. Infections were recorded when documented in the bloodstream, central nervous system, and lungs.

Statistical Analyses

Categorical patient and disease characteristics, including transplant indication, donor and recipient CMV status, sex, and stem cell source were compared by type of donor (RD versus URD) using Fisher's exact test (Table 1). Fisher's exact test was also used to compare the time-specific rates of severe acute GVHD and extensive chronic GVHD. A nonparametric Wilcoxon test was used to compare age at transplant among patients with RD versus URD donor transplants. Linear repeated-measures models were used to estimate mean cell counts including CD3, CD4, CD8, natural killer (NK) cells (CD16/56) and CD19, and linear contrasts to test for difference between patients with RD versus URD transplants at 100 days, 6 months, and 1 year post-HSCT. Power transformations (eg, log) were used to improve the fit of these models. The odds of bacterial or viral infection were calculated using generalized estimating equations models, which accommodate more than 1 observation (time point) per patient.

RESULTS

Patient Characteristics

Of 91 patients transplanted, 71 (78%) met inclusion criteria for this analysis. Four patients died before engraftment, 9 had graft rejection and autologous recovery, and 7 patients were excluded for lack of full immune reconstitution data. Thirty-nine received RD and 32 received URD stem cell products (Table 1). Indications for transplantation included bone marrow failure syndromes, hemoglobinopathies, immune dysregulation, and metabolic/genetic defects. There were no

Table 1
Patient Demographics and Transplant Characteristics

Characteristic	Related donor	Unrelated donor	*P* value
Disease			.50
Bone marrow failure	9 (23%)	8 (25%)	
Hemoglobinopathy	18 (46%)	11 (34%)	
Immune dysregulation	10 (26%)	8 (25%)	
Metabolic/genetic	2 (5%)	5 (16%)	
Sex			.99
Male	23 (59%)	19 (59%)	
Female	16 (41%)	13 (41%)	
Median age (range in years)	8 (0.2-20)	2.5 (0.08-20)	.016
Stem cell source			.40
Bone marrow	34 (87%)	27 (84%)	
Bone marrow + Cord	2 (5%)	0 (0%)	
Peripheral blood	3 (8%)	5 (16%)	
HLA match			
Matched	28 (71.8%)	18 (56.3%)	
8/10	0 (0%)	3 (9.4%)	
9/10	1 (2.6%)	4 (12.5%)	
7/8	1 (2.6%)	3 (9.4%)	
6/6	9 (23%)	3 (9.4%)	
5/6	0 (0%)	1 (3%)	
CMV status of donor and recipient			.027
Donor negative; recipient negative	15 (39.5%)	13 (40.7%)	
Donor negative; recipient positive	6 (15.8%)	5 (15.6%)	
Donor positive; recipient negative	2 (5.2%)	9 (28.1%)	
Donor positive; recipient positive	15 (39.5%)	5 (15.6%)	

Values are presented as median (range) or number (%).

differences in transplant indications between RD and URD HSCT. RD transplant recipients were significantly older (median age 8 years; range 0.2 to 20) than those who received URD HSCT (median age 2.5 years; range 0.08 to 20; [P =.016]). CMV+ donors into CMV− recipients were significantly more common among patients who received URD HSCT (P = .027). There was no significant difference in sex or stem cell source between the 2 groups.

GVHD

Nineteen patients developed grade II to IV acute GVHD by 6 months post-transplantation (26.7%). Fourteen patients had grade III to IV acute GVHD (1 RD; 13 URD). At 1 year post-transplantation, 21 patients had chronic GVHD (4 RD; 8 URD), with 12 patients having extensive chronic GVHD.

Immune-Reconstitution

NK cells

Early NK cell (CD16/56) recovery was noted with mean values within normal limits by day 100 post-HSCT (164 cells/μl and 203 cells/μL, respectively, in RD and URD). There was no significant difference between the RD and URD groups at any of the time points post-HSCT (Figure 1A; P = .19).

T-lymphocytes

Normal mean CD3, CD4, and CD8 T-lymphocyte numbers were reached in the RD group by 6 months and in the URD group by 1 year. Mean CD3 T-cell values were significantly higher in patients who received RD HSCT at 100 days and 6 months post-HSCT compared with URD donor HSCT recipients (Figure 1B; P = .035 and .048, respectively). Similarly, mean CD4 and CD8 T-cell values were significantly higher in patients who received RD versus URD HSCT at 6 months and 1 year post-HSCT, respectively (Figure 1C-D; mean 322.1 cells/μL compared with 185.3 cells/μL at 6 months [P = .014] and 713.3 cells/μL compared with 403.8 cells/μL at 1 year [P = .025], respectively).

B-lymphocytes

The pace of CD19 B-cell recovery was markedly different between RD and URD groups. Normal levels were achieved in the RD group by 100 days and in the URD group by 1 year post-HSCT. CD19 cells were significantly higher at all time-points in the related group (Figure 1E; P = .0002 at 100 days, <.001 at 6 months and 1 year).

Immunoglobulin levels at 6 months and 1 year for all patients are shown in Table 2. Levels reflect the lag in recovery of B cell numbers.

Infections

Infections were recorded per interval period: 0 to 3 months (100 days), 3 to 6 months, and 6 to 12 months (1 year) post-HSCT for all patients meeting inclusion criteria (Table 3). There was no statistically significant difference in infection rates (P = .13), bacterial infections (P = .29), or viral infections (P = .25) between the RD and URD groups. Thus individual time points were not modeled for statistical significance. Fungal infections only occurred after URD HSCT; in 1 patient before day +100 and in 3 patients between 6 and 12 months as described below.

Infections occurred predominantly in the early post-transplantation period. During the first 100 days, 15 patients (39%) who received an RD transplant and 9 (28%) who received a URD transplant experienced a total of 44 bacterial infections. The most common organisms included Coagulase-negative *Staphylococcus* species (n = 16), *Enterococcus* species (n = 6), and *Klebsiella* species (n = 3). Viral reactivation was noted during the first 100 days in 32 patients; 21 (53%) after RD HSCT and 11 (34%) after URD HSCT, and included CMV (n = 16), human herpesvirus-6 (n = 5), Adenovirus (n = 4), EBV (n = 8), BK virus (n = 1), and herpes simplex virus (n = 2) (Figure 2B). One patient had a presumed fungal infection based on elevated Beta-D-glucan assay in the first 100 days and was successfully treated with micafungin.

At 6 months post-HSCT, the incidence of infections decreased. Four (10%) RD HSCT and 8 (25%) URD HSCT recipients had 22 bacterial infections, with the most common organisms being coagulase-negative *Staphylococcus* species (n = 4) and *Enterococcus* species (n = 4). Fourteen patients had viral reactivation, 4 (10%) after RD and 10 (31%) after URD HSCT with the most common viruses being EBV (n = 5) and CMV (n = 4). There were no fungal infections documented in either group. The only infection-related death in this cohort occurred in a patient with aplastic anemia who received a URD HSCT and died of disseminated adenovirus infection and grade IV acute GVHD on day +173.

A further decrease in the incidence of infections was observed in the period between 6 and 12 months post-HSCT. During this 6-month interval, 2 (6%) RD and 5 (16%) URD transplant recipients had 11 infections, with the most common organism being coagulase-negative *Staphylococcus* species (n = 4). Eight patients, 1 (3%) patient who received an RD and 7 (22%) who received a URD HSCT, tested positive for viruses, with the most common being human herpesvirus-6 (n = 4) and EBV (n = 4). Three patients had fungal infections during this time period, with *Candida* sp. (n = 2) and *Aspergillus* sp. (n = 1). All were successfully treated.

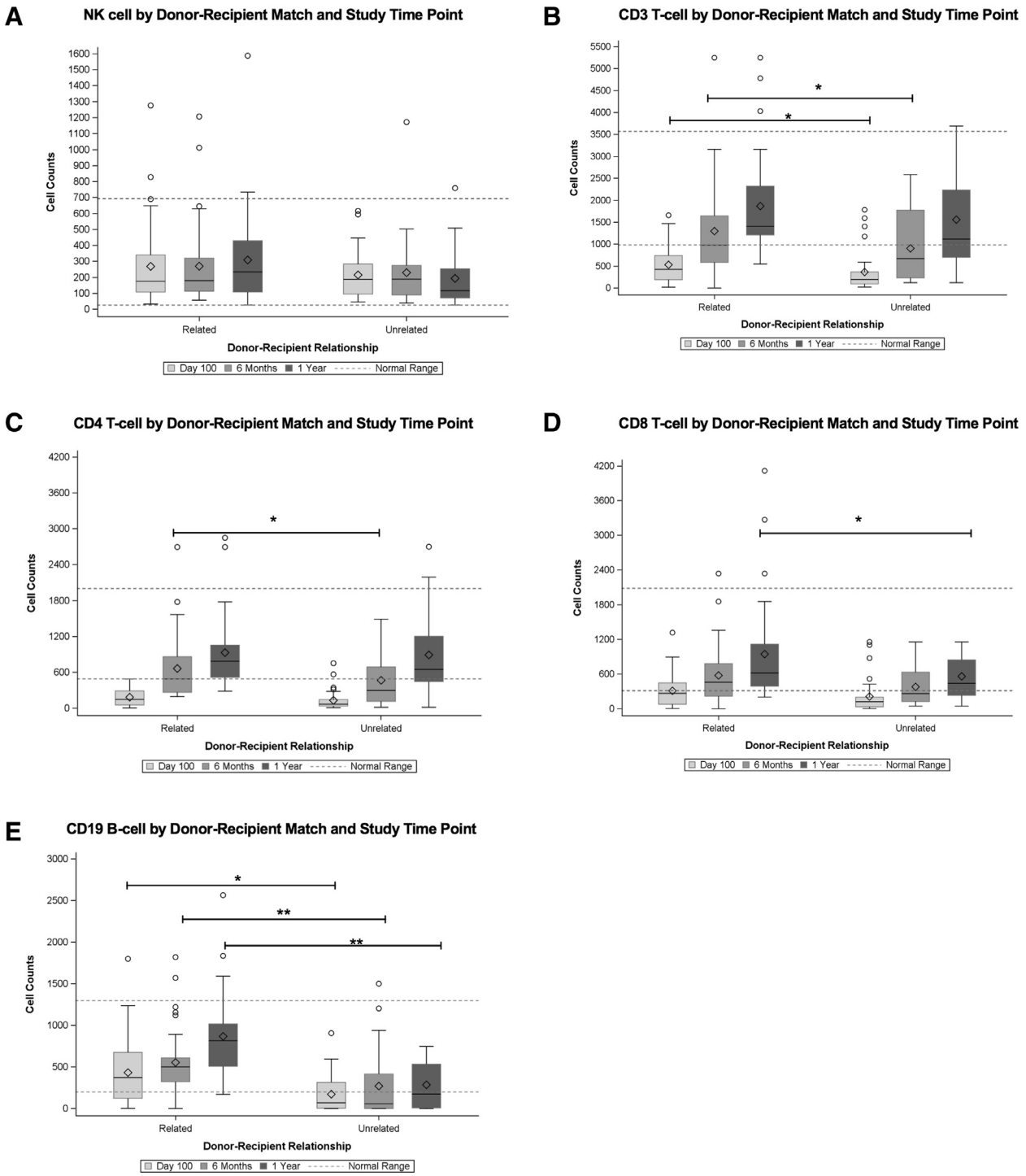

Figure 1. Immune reconstitution post-transplant. (A) CD16/56 NK cell, (B) CD3 T-cell, (C) CD4 T-cell, (D) CD8 T-cell, and (E) CD19 B-cell counts at 100 days (light gray), 6 months (gray), 1 year (dark gray). Dotted lines indicate normal range. Asterisk (*) indicates $P < .05$, double asterisk (**) indicates $P < .001$.

DISCUSSION

The kinetics of immune reconstitution and infection patterns are an important parameter of recovery after immunosuppressive RIC and HSCT in children. The preparative regimen we used included early alemtuzumab during conditioning (day −21 to −19) to maximize host immune suppression and minimize in vivo donor T-cell depletion to facilitate engraftment. This strategy was also hypothesized to allow early immune reconstitution post-transplantation and thus decrease the serious late infectious complications previously described with alemtuzumab [13]. Immune reconstitution was evaluated separately after RD and URD donor transplants due to potential variability in immune recovery between the groups. There was no difference in sex, primary disease, or stem cell source between the 2 groups. However, the median age in the RD group was significantly greater than the URD group and could have contributed to differences noted in immune recovery. Additionally, although the intensity and duration of GVHD

Table 2
Immunoglobulin Levels at 6 Months and 1 Year Post-Transplant

	6 months		1 year	
	No.	Median (Range)	No.	Median (Range)
Immunoglobulin A (mg/dL)	55	53 (5.8-755)	52	51.75 (0.52-232)
Immunoglobulin M (mg/dL)	63	60.9 (1-403)	59	51.5 (1-256)
Immunoglobulin G* (mg/dL)	57	707 (90.7-1980)	52	721.5 (4.8-1910)

* Intravenous immunoglobulin prophylaxis was not included as a transplant recommendation; N=number of patients

Table 3
Incidence and Timing of Infections After HSCT

	Time post-HSCT					
	100 days		6 months		1 year	
	URD	RD	URD	RD	URD	RD
Bacterial	9 (28%)	15 (39%)	8 (25%)	4 (10%)	5 (16%)	2 (6%)
Viral	11 (34%)	21 (53%)	10 (31%)	4 (10%)	7 (22%)	1 (3%)
Fungal	1 (3%)	0 (0%)	0 (0%)	0 (0%)	3 (9%)	0 (0%)

The number of infections for each group of pathogens is listed by donor type.

prophylaxis/treatment between the 2 groups may have varied, we found that T-cell immune reconstitution was robust beyond day 100 post-HSCT after both RD and URD transplants with this regimen. B-cell reconstitution was delayed after URD compared with RD HSCT, but this did not translate into a significantly increased incidence of late bacterial infections in the former group. The small numbers (N = 5 when considering 8/8 donor recipient pairs fully matched) precluded analysis of mismatched transplants separately. This subgroup could require prolonged immune suppression because of the HLA mismatch and consequently have delayed immune reconstitution compared with matched URD transplant recipients. Because of early infection risks with an immunosuppressive conditioning regimen, exclusion criteria to enrollment included the presence of active uncontrolled invasive infections. In addition, patients received infection prophylaxis with ciprofloxacin and a broad-spectrum antifungal agent for 100 days after HSCT. This strategy was successful in avoiding infection-related mortality from bacteria and fungi.

In keeping with immune recovery patterns, the highest risk period for infections was during the first 100 days post-HSCT, with 33.8% of patients having a bacterial infection. The incidence of infection decreased to 16.9% between 3 and 6 months, and 9.9% between 6 months and 1 year. There was no significant difference in the incidence of infections between RD and URD transplant recipients. Fungal infections occurred in 5.6% of patients. Only 1 patient had an early infection. Late fungal infections (N = 3) were noted in patients who received URD HSCT and developed extensive chronic GVHD; a known at-risk population. They were all successfully treated with antifungal therapy. Viral infection is also of concern after T-cell-depleting serotherapy, and antiviral prophylaxis is not beneficial against the majority of invasive viruses. The rates of viral reactivation followed a similar pattern to bacterial infections, with the highest risk period being in the first 100 days (45.1%) and decreasing at 6 months and 1 year (19.7% and 7.0%, respectively). The rate of adenovirus infection was 11%, and 1 death was attributed to adenovirus. CMV reactivation occurred in 18% of patients. However, there was no CMV-related mortality, presumably because of regular surveillance and pre-emptive therapy. As in bacterial infections, there was no significant difference in the incidence of viral infections between RD and URD transplant recipients.

Immune reconstitution is rarely reported after HSCT in children. Koehl et al [19] reported lymphocyte recovery in 32 pediatric patients undergoing HSCT for malignancy after primarily MAC and described CD4 and CD8 reconstitution between 3 and 6 months post-transplantation. Delay in lymphocyte recovery beyond the first year was associated with significant morbidity and mortality [19]. Jimenez et al [24] compared immune reconstitution after MAC and RIC conditioning (with no in vivo T-cell depletion) in patients 17 years of age and older. Recovery of CD3 T-cells (median $\geq$1000 cells/μL) occurred in the RIC group by 9 months, earlier than in the MAC regimen in the absence of lymphodepleting serotherapy. However, in variance with our experience, although CD8 T cells recovered in their patients early, the regimen did not support recovery of CD4 T-cells ($\geq$500 cells/μL) in the same time period [24,25]. The role of serotherapy when used to mitigate GVHD is of further concern in relation to delayed immune reconstitution and risk of infection. When MAC and RIC patients were evaluated for immune reconstitution in patients who were 15 years or older, those who received RIC and serotherapy took over a year to reconstitute CD3, CD4, and CD8 T cells in a report by Saito et al [26]. Similarly, immune reconstitution after serotherapy using antithymocyte globulin or alemtuzumab in 148 pediatric patients reported by Willemsen et al [20] revealed a slow pace of immune reconstitution and T- and B-cell recovery with normal numbers achieved at or beyond 1 year post-HSCT. Compared with these reports, the relatively rapid pace of immune reconstitution we noted is likely related to the “early” timing of the alemtuzumab in keeping with our initial hypothesis. A similar pattern of earlier recovery has been shown after cord blood transplantation using early antithymocyte globulin serotherapy [27]. The timing of serotherapy and dose must be carefully considered to balance the risks of delayed immune reconstitution with GVHD and graft rejection.

The pace of immune reconstitution is directly related to infection risks, and delayed viral or fungal infections can be detrimental when patients are not as closely monitored as in the early post-HSCT period [4,13]. Timely immune recovery of CD4 T cells as was noted in our patients, which helped avert these late infections, especially important as children return to school and other infection-vulnerable locations during this time period. CD19 B-lymphocyte recovery has been traditionally described 3 to 5 months post-transplantation [19,26]. However, delayed B-cell recovery is a known complication of alemtuzumab. In our series, CD19 B-cell numbers recovered in timely fashion in patients receiving RD HSCT (by day 100 post-HSCT) but was delayed in the URD group and was also reflected in immunoglobulin levels. In both groups (RD and URD), however, the incidence of infections declined in parallel with time. Nonetheless, given the delayed recovery of B-cells, we support the need for continued surveillance for bacterial infections in the first year post-HSCT.

Our fungal and viral infection risks compare favorably with those previously described with RIC regimens [28,29] Satwani et al [28] found a trend toward higher rates of fungal infections in RIC compared with MAC (18% and 9%, respectively), whereas Fukuda et al [29] described fungal-infection-related mortality rates of 9% after a RIC regimen. There was no mortality related to fungal infections in our cohort. Similarly, Chakrabarti et al [13] reported a high incidence (46.6%) of CMV reactivation, many of late onset, after alemtuzumab use in the immediate pre-HSCT period to decrease GVHD. Other reports have

described CMV reactivation rates between 15% and 31% with in vivo T-cell depletion [28,30] Similarly, the incidence of adenovirus infections after allogeneic HSCT is 3% to 27% [28]. Adenovirus and CMV infection or reactivation rates of 11% and 18%, respectively, in our cohort compare favorably with these prior reports, as does the rapid decrease in infections after day 100 post-HSCT. The incidence of early bacterial infections (34%) that we observed was comparable with previous reports and likely related to the conditioning regimen as it occurred across disease categories [6,31] Although there was no associated mortality, this complication requires close surveillance and prompt therapy.

This report describes the correlation between numeric recovery of lymphocyte subsets and infection patterns in children with NMD transplanted using an alemtuzumab based host immunosuppression. It highlights the duration of the risk period for bacterial, viral, and fungal infections and demonstrates a decline in these complications after the first 100 days post-HSCT. The rapid decline in susceptibility to infections and the favorable T-cell immune reconstitution with “early” alemtuzumab allows for timely reimmunization and safe reintegration of pediatric patients into school and society.

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